2nd ENLARGED EDITION

M.M. SAXENA

ENVIRONMENTAL ANALYSIS WATER, SOIL AND AIR

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PREFACE TO THE FIRST EDITION

Efficient monitoring of kinds, ranges, and variations of various environmental factors in space and time is one of the prerequisites for all environmental studies and practices. Methodological literature on the analysis of abiotic and biotic parameters of different environments (viz., water, soil and air) is so scattered that a worker in this field is often perplexed in search and choice of right method. There is hardly any far-flung literature to cover the problem in its totality. In the present attempt, I have endeavoured to present the analytical methods for a wide array of physical, chemical, and biological factors pertaining to water, soil, and air environments, within a single pair of covers.

The subject matter of the book is divided into Sections A, B, and C dealing with water, soil, and air environments respectively. Each section comprises two chapters, one on sampling techniques and the other on analytical methods. Each chapter on analysis is subdivided to deal with physical, chemical, and biological parameters of respective environment separately. A brief account on each parameter is given which provides its environmental significance.

Although a large number of environmental factors are tried to be covered still these cannot be regarded as the ultimate. Methods presented in this book are not claimed to be my original, but are taken from various authentic sources to which I am indebted. References to the authors are not made in the text but a bibliography is included at the end. Modifications in the methods and calculations are made wherever felt necessary in order to make them simple to follow and apply. Not all the methods given here represent to the latest of available ones but the selection is made on the criteria of practicability and ease at no or minimum cost of precision of result. Chemical reactions and principles involved are deliberately not included so as to avoid complexity of matter. Identification of flora and fauna being a vast subject in itself, could not be incorporated in the limited size of the book.

Thanks are due to Messrs Agro Botanical Publishers (India) for their cooperation.

October 2, 1987 **M M Saxena**

PREFACE TO THE SECOND EDITION

The overwhelming response to the first edition of the book that led to its running out of print in this short duration has encouraged to bring out its second edition. Critical views of readers and reviewers have helped in developing the book for which I am indebted to them.

Standards for drinking water as prescribed by World Health Organization and Ministry of Works & Housing, and those for disposal of wastewater as prescribed by Indian Standards Institute have been included. Minimum National Acceptable Standards (MINAS) for the effluents of various industries have also been appended.

I am confident that the users will find the book more useful and apprise of their critical views for further improvement of the book.

October 2, 1989 **M M Saxena**

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Chapter 1

ENVIRONMENT AND ITS ANALYSIS

1.1. THE ENVIRONMENT

The term **environment** in its broader perspective may be defined as what that surrounds a living or a nonliving object. The modern functional approach to environment recognises the fact that the environment of an object is not necessarily the same as that of the area in which the object is found. Organisms occupying the same general environment may in fact be living under different sets of conditions. To make the concept more clear, the functional environment of an organism is not the generalized environment in which it occurs, but is that which comprises continuously changing complex of all conditions and influences, which are interacting with that organism (Platt & Griffiths, 1964). Many of the workers have used the term **microenvironment** to designate a functional environment, that is the specific environment of specific organisms. Literally, however, the term microenvironment refers to a small environment, and since it is a relative term, it may signify the immediate environment of an organism.

An environmmental factor measured for ambient air on land may be relevant to surface dwellers but not to the soil or cave organisms. In same manner, the set of environmental conditions in the surface water may not be the same for lower profiles. As such, in all cases functional environment and its interrelationships with the object under study should be explored. For holistic environmental surveillance a series of measurements in vertical and horizontal gradients have far greater significance than single point measurements. Not only in space, but variations in time (diel, seasonal, annual or geological etc.) are equally important in a dynamic functional environment.

1.2. ENVIRONMENTAL ANALYSIS: WHY?

Although the history of civilization is as old as the man's knowledge of fire and tools to alter his environment, but its wholesome impact on nature has never been so grave as during recent decades. The present day speedy socio-economic development of humanity, which is the highest form of vital activity, has most vigorously stimulated changes in nature. Technological advancement, if on one hand, has bettered the human life on this planet, it has sharply intensified the pollution of environment and posed still severe threats for future generation, on the other hand. Increasing population growth, extermination of a number of life forms because of man's activity, dwindling natural resources that man has been consuming for centuries, and deterioration of overall environmental quality, are the primary ecological problems that man is creating and facing today. This renders it undisputed that there is an acute need to develop an ecological approach for all development planning.

The evaluation of natural environment and of damage to it through human and non-human agencies is essential in the light of preservation of aesthetic values of nature, conservation and management of natural resources, and amelioration of ecological crises. Successful pollution abatement, in the words of Odum (1971), of course, depends not only on treatment and control but also on efficient monitoring of the general environment so that we will know for sure when control measures are needed and whether those in operation are working. Thus a thorough understanding of structural and functional attributes of the environment and their spatial and temporal gradients is essential to develop a rational and scientifically managed relationship between man and the nature, so that the human ingenuity can avoid the impending ecological catastrophes.

1.3. ENVIRONMENTAL ANALYSIS: HOW?

Ecological research no longer confines to mere qualitative approach but is now firmly established on a quantitative basis. As has earlier been mentioned, the spatial and temporal variations in structural and functional attributes of environment, which may be categorized as physical, chemical, and biological, have to be monitored both qualitatively and quantitatively in order to have a comprehensive evaluation of an ecosystem. The biosphere may be divided into three components viz., hydrosphere (water), lithosphere (earth), and atmosphere (air), and continuous exchange of matter takes place among these components and the organisms. In the oncoming chapters sampling and monitoring methods for a wide array of environmental factors of water, soil, and air are delt under Sections A, B, and C respectively.

Section A

Chapter 2

WATER SAMPLING

Knowledge of morphometric details about the body of water in question,planned sampling stations within the body, and use of suitable methods for collection, handling and preservation of sample for parameter(s) in question are the subjects of prime consideration for the successful monitoring of an aquatic ecosystem.

2.1. MORPHOMETRY

The study that deals with measurement of morphological features of the basin of a body of water and its included water mass is known as **morphometry.** Beacuse many fundamental ecological conditions are directly dependent upon structural relations of water it is necessary to make measurements of various morphological features. Following general and morphometric information should be generated before studying structural and functional attributes of the system.

GENERAL INFORMATION:

i. **Type:** The type of body of water viz.,lake, pond, marsh, swamp, well, stepwell, spring, stream, river, estuary, or sea etc. should be noted.

ii. Location: The locality, latitude, longitude, and altitude at which the study area is situated should be noted from authentic maps or topo-sheets.

iii. Historical background: Collect the information pertaining to geological history of the basin and surroundings of natural waters. For atrificial bodies the construction or excavation details are of importance. Past and present human interactions with the study area should also be noted.

iv. General physiography: Salient physiographical features related to basin, bank, and catchment area of the body of water should be noted. Some characters descriptive of such features are as follows: for basin- bed rock, boulders, coarse gravel, fine gravel, hard pan, intermixture, miscellaneous debris, mud, muck, marl, peat, rubble, sand, silt, clay, and false bottom etc.; for bank and catchment- cliffs, cultivated, shrubs, wooded, barren, eroding, high abrupt, high sloping, low, marshy, swampy, boggy, rock outcrop, and topsoil etc.

For inland surface waters following **morphometric parameters** are of great importance, however, some of them can be applied to ground and costal waters too.

i. Area: The surface area of water-spread can be calculated from a shore-line map of the body of water.

ii. Bathymetry: A bathymetric or contour map is one which denotes the depth at different points in the body of water.

iii. Maximum length: It is the length of line connecting two most remote extremities of the body of water.

iv. Maximum effective length: It is the length of line connecting two most remote extremities of the body of water along which wind and wave actions occur without any kind of interruption (viz., islands or their groups). Maximum length and maximum effective length may be, and in most cases are, identical.

v. Maximum width: It is the length of straight line connecting most remote transverse extremities of a body of water.

vi. Maximum effective width: It is the length of straight line connecting most remote transverse extremities of a body of water along which wind and wave actions occur without any kind of land interruption.

vii. Mean width: It is equal to the area divided by maximum length.

viii. Depth: It is the vertical distance between the surface and the underlying bottom.

ix. Maximum depth: It is the depth measured at the deepest point.

x. Mean depth: It is calculated by dividing the volume of the body of water by its surface area.

xi. Volume: It is derermined by computing the volume of each horizontal stratum as limited by many submerged contours on the bathymetric map. The sum of all such strata represents the total volume of the body of water.

In order to compute the volume of one horizontal stratum, say the uppermost, the area of surface and first submerged contour are taken into account in following formula.

$$\text{Volume } (m^3) = \frac{h}{3} \ (a_1 + a_2 \ + \sqrt{a_1 \ a_2})$$

Where, h = depth of stratum in question (m); a_1 = area of upper surface of stratum (m^2); and a_2 = area of lower surface of stratum (m^2).

2.2. SETTING UP OF SAMPLING STATIONS

No standard lines or directions can be followed for setting up of sampling station in a body of water for all the purposes. The selection of sampling stations is decided in the light of objectives of environmental monitoring programme.

For simple characterization of water a few random samples taken from surface during morning hours may be sufficient, but for a thorough investigation, or an investigation with something specific in mind, the sampling stations and programme have to be planned beforehand. A bathymetric map of the study area with details such as points of inflow, outflow, pollution, human activity etc., if any, is very useful for this purpose.

In large bodies of water, like a lake, the stations should be so planned that they represent whole of the body, taking specially into consideration the shallow and deep regions, points of inflow, outflow, and human activity etc. For a small shallow pond, however, sampling at only the bank region may suffice. In deeper waters it is advisable to take samples from minimum of three (surface, mid-depth, and bottom) or more depth-wise samples from each of the stations fixed, while in shallow waters sampling from only surface and bottom may be sufficient. In lotic waters the stations are fixed along the length of the stream, and also along the width when the stream is wide enough. When a body of water is polluted by a point source the stations should be set in such a plan that they represent water at and off the pollution source. In organically polluted running waters atleast one sampling station should be fixed above the outfall of the wastes (clean water) and a number of stations (atleast four) downstream representing the polysaprobic, mesosaprobic, oligosaprobic, and clean water (recovered) zones. Accessibility, time schedule, and manpower are the other important considerations in the selection of sampling stations.

A sampling station may be marked by fixing a pole with flag or placing an anchored float in water.

2.3. COLLECTION OF WATER SAMPLE

For collection of sample trom surface a plastic bucket, jug, or bottle (glass or polyethylene) may be used. Samples from the bottom of shallow water can be collected by lowering a closed glass or polyethylene bottle to the bottom, opening and closing it there by hands, and taking out. But for collecting the subsurface samples in deeper water various types of water samplers are used, the common ones in use today are Von Dorn water sampler, Ruttner water sampler, Dussart-flask water sampler, thermos-flask water sampler, and pump samplers. Before use, it is always desirable to rinse the water sampler thoroughly with the water to be collected by it.

i. Von Dorn water sampler (Fig.1): This is one of the most common type of water samplers. It is made up of hollow cylinder of PVC having

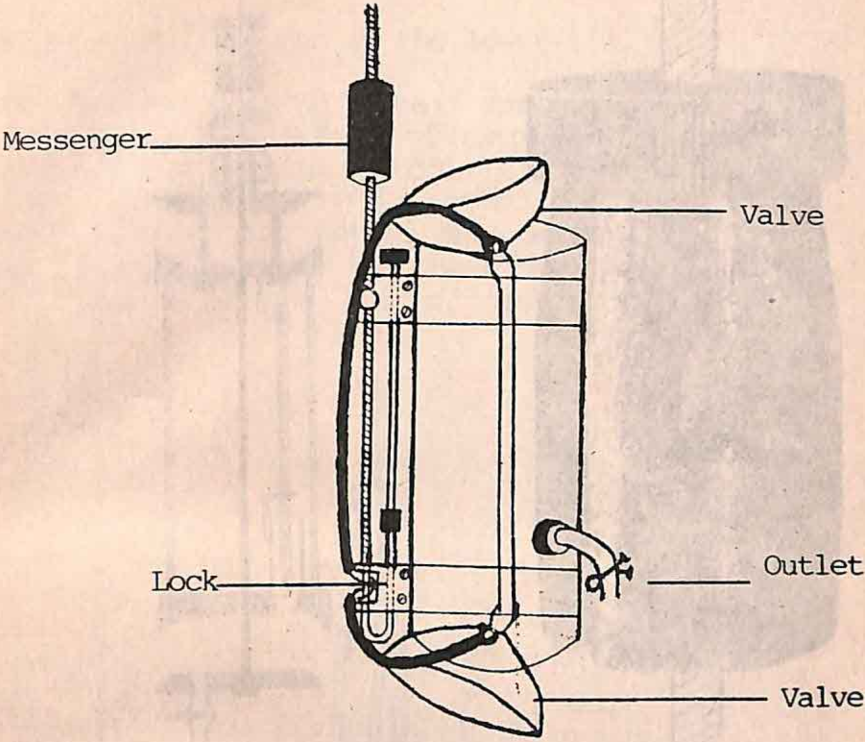

Fig. 1. Von Dorn water sampler.

two interconnected (by rubber tube) rubber valves on its open ends. The two valves are pulled out and their strings are attached to a lock fitted outside the cylinder. This keeps the cylinder of sampler open at both the ends. The sampler in this condition is lowered, with the help of a graduated rope, to the desired depth and a metallic messenger (Fig. 2) is released along the rope which strikes the lock and releases two valves to close the sampler. The sampler is pulled out of water with the sample of desired depth in it, which can be taken out through a tube provided on the side for this purpose.

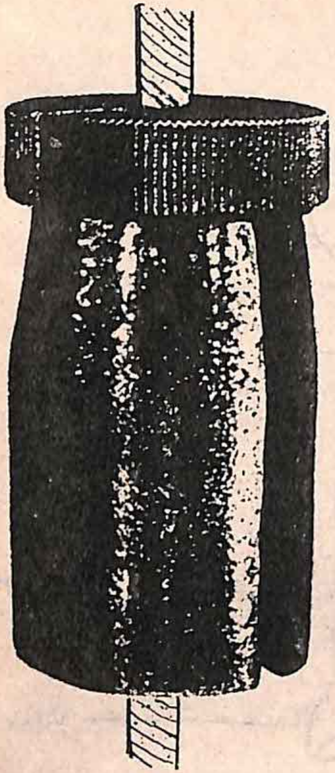

Fig. 2. Messenger weight.

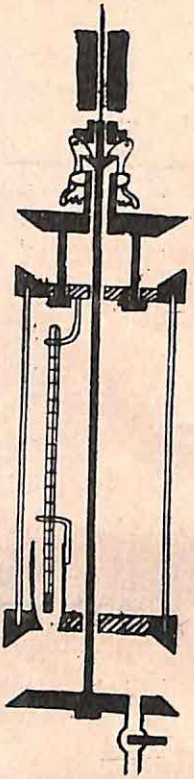

Fig.3. Ruttner water sampler.

ii. Ruttner water sampler (Fig.3.): It is made up of a cylinder of plexiglass having on its two open ends the lids interconnected by a central shaft. The two lids are held open with the help of a trap mechanim provided on the upper part of the shaft. The sampler is lowered in water to the desired depth with the help of a graduated rope and, as in case of Von Dorn water sampler, a mesenger is released along the rope to open the trap and allow two lids to close the sampler. It is taken out of water and sample is drained through a tap provided in the lower lid.

iii. Dussart-flask water sampler (Fig.4): It is a very simple type of sampler. It consists of a glass bottle with a weight tied to its bottom and a graduated rope to its neck. The cork fitted to the mouth of the bottle has two holes holding glass tubes, one short and the other long reaching up to bottom of the bottle. The outer ends of

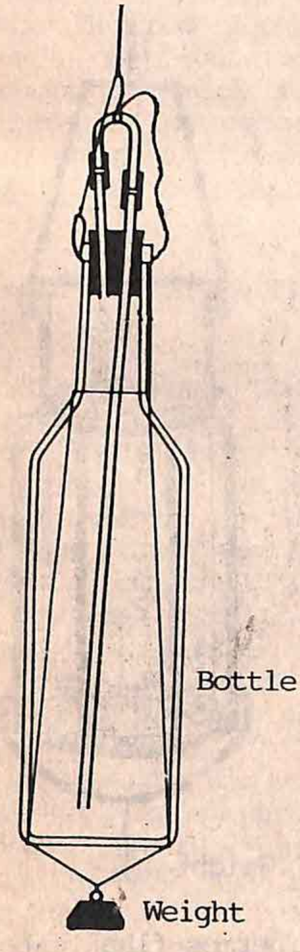

Fig. 4. Dussart-flask water sampler.

the two tubes are interconnected by a rubber tube which is tied to the main rope in such a way that by a slight jerk it can be pulled out. The sampler is lowered in water and with a jerk to the rope the rubber tube is pulled out. As a result water enters through the longer glass tube displacing out the air through shorter tube. The bottle is pulled out of water with sample in it.

iv. Thermos-flask water sampler (Fig.5):

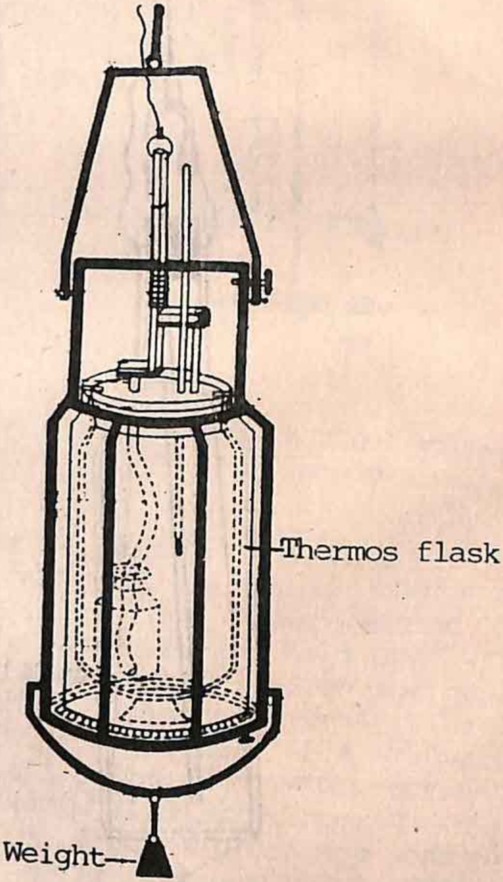

Fig.5. Thermos-flask water sampler.

This sampler consists of a thermos-flask having a cork at its mouth fitted with a thermometer. It has two cords, one to hold the sampler and the other to open and close the cork. This sampler has an advantage over others that sample in it is brought to the surface in unmodified thermal condition, however, its double cord operation is an disadvantage because the two cords may get interwined and make the operation difficult.

v. **Pump samplers:** In this type of samplers a weighted and graduated rubber or plastic tube is lowered to the desired depth. Through this tube a pump sucks a continuous stream of water. The peristaltic pumps are more suitable for this purpose because there is no contact between metal and water. The advantage of this type of sampler is that large samples may be obtained with causing little disturbance.

2.4. HANDLING AND PRESERVATION OF WATER SAMPLE

Parameters like temperature, pH, free carbon dioxide, alkalinity, dissolved oxygen (by oxygen meter), and sulphide should immediately be recorded or estimated after collecting the sample. Dissolved oxygen if to be estimated by Winkler's method should immediately be fixed by adding manganous sulphate and alkaline potassium iodide solutions. For most other physical and chemical analyses some holding time for samples is permissible. Sample from the sampler should be transferred to well rinsed, appropriately labelled, suitable sample containers (bottles). Containers made up of borosilicate glass, polyethylene, or polypropylene are suitable for this purpose; never use a metallic container. Light weighted, chemically inert, unbreakable, and low cost bottles of polyethylene or polypropylene are considered to be the better choice over those made up of borosilicate glass which are heavy, breakable, and very expensive. However, for the analyses of parameters like dissolved oxygen, oil and grease the use of glass bottles is recommended.

The label on the sample container should clearly denote the following information: Name and location of body of water; Date and time of sampling; Station No.; and Depth.

The samples in well labelled and tightly capped containers should be brought to the laboratory in an ice-box and kept in a freezer to check the biological activity and preserve them (cryopreservation). For estimation of metallic ions a sample may also be preserved by acidification (pH 2 to 3) by sulphuric acid but in such cases conditions of alkalinity, conductivity, and sulphate etc. are greatly altered. Mercuric chloride @ 50 mg per litre of sample is good preservative to check the biological activity but on the other hand it interferes in the analyses of various nitrogen forms and chlorides. The long term cryo or chemical preservation of sample may lead to alterations in the chemical nature of the sample, hence it is advisable to carry out the analyses in field or in laboratory as early as possible after collection.

2.5. COLLECTION, HANDLING, AND PRESERVATION OF BIOLOGICAL SAMPLES

Techniques for collection, handling, and preservation of biological matter of water (viz., macrophytes, phytoplankton, zooplankton, periphyton, benthos, and microconsumers etc.) vary greatly because of the diversity of life forms. Such details are discussed in Chapter 3 (3.3).

Chapter 3

WATER ANALYSIS

3.1. PHYSICAL PARAMETERS

3.1.1. Movement

Natural waters remain partly or wholly in motion due to action of various factors like wind, in or out flow, and silt etc. These movements affect the transfer of matter and energy in the system. In polluted waters currents have important role in the process of self-purification.

I. Pitot tube method

Material
Pitot tube: It is a simple L-shaped glass tube open at both the ends (Fig.6). Its one end is shorter than the other. Longer axis of the tube may be graduated in centimetre or its fractions.

Method
Dip the Pitot tube vertically in flowing water, with the shorter end submerged and facing the flow. Water enters the open end and rises a distance

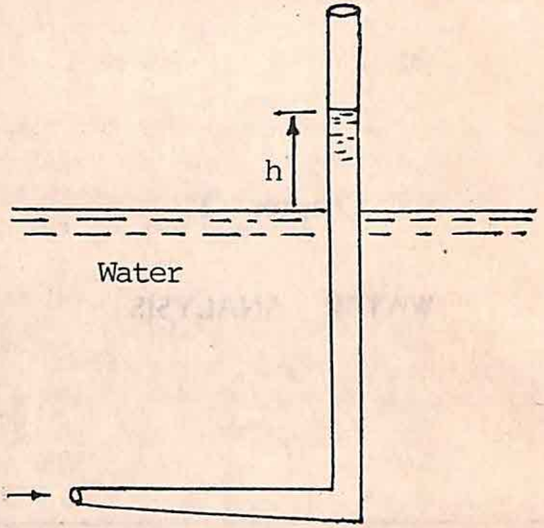

Fig.6. Pitot tube.

h above water level outside in the vertical limb of the tube. Measure the distance **h.**

Calculation

$$v = 0.977\sqrt{2gh}$$

Where, v = velocity (m/sec); 0.977 = an average constant; g = acceleration of gravity (9.8 m/sec); h = height of water column (in fractions of a metre).

To take capillarity into account, dip the upper end of the tube into water and measure the rise of water due to capillarity. Subtract this value from **h.**

II. Float method

Material

i. Float (cork or wood); ii. magnetic compass; iii.

stop watch; and iv. measuring tape etc.

Method

Release a float on the surface of water and note the direction of flow of water with the help of a magnetic compass. Fix two poles at a known distance (d) in the direction of flow. Now release the float at one pole and note the time (t) taken in reaching to the other pole using a stop watch. Repeat the exercise for several times and calculate the mean of time.

Calculation

$$v = \frac{d}{1.2\ t}$$

where, v = velocity (m/sec); d = distance between the two poles (m); 1.2 = a constant; and t = time (sec).

III. Flow meter method

Modern flow meters are very convenient and accurate instruments for measurement of flow of water. These can be lowered at any depth of water to measure the flow at that level. Flow meters are also attached with plankton net to know the actual amount of water passed through the net.

3.1.2. Colour

By **colour** in water means those hues inherent within the water itself which result from colloidal substances and materials in solution. In natural waters, colour may occur due to the presence of humic acids, fulvic acids, metallic ions, suspended matter, phytoplankton, weeds, and industrial effluents.

I. Platinum - cobalt method

This method gives a quantitative measure of the hue.

Material

i. Nessler tube, 50 ml; and ii. colour standards: dissolve 1.245 g of potassium chloroplatinate and 1.0 g of crystalline cobaltous chloride in a little distilled water; add 100 ml of concentrated hydrochloric acid and dilute to 1 l with distilled water. This solution has a colour value of 500 colour units. Prepare standards having colour value of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70 by diluting 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 ml of above solution with distilled water to 50 ml in standard Nessler tubes. Indicate on each tube the colour value, and protect them from evaporation and contamination.

Method

Centriguge the sample at high speed to remove suspended matter. Fill standard Nessler tube with sample to same level as that of standard (50 ml). Compare the colour of sample with that of various standard tubes held vertically above a white surface and find one standard which has same hue as that of sample and read its colour value.

If the sample shows a colour greater than 70 unit, it should be diluted with distilled water. In such case, original colour is computed as follows.

Calculation

Colour (unit) = estimated colour x dilution factor

II. Forel-Ule colour scale method

This method gives a qualitative recognition of the colour of sample.

Material

i. Solution A: Add 0.5 g of $CuSO_4.5H_2O$ to 5 ml strong NH_4OH and dilute to 100 ml with distilled water;

ii. Solution B: Add 0.5 g of $K_2CrO_4.5H_2O$ to 5 ml of strong NH_4OH and dilute to 100 ml with distilled water; and

iii. Solution C: Add 0.5 g of $CoSO_4.7H_2O$ to 5 ml of strong NH_4OH and dilute to 100 ml with distilled water.

Empirical Forel-Ule colour scale is prepared by mixing different proportions of the above solutions (solution A,B, and C) as shown in Table 1.

Method

Centrifuge the sample so as to remove the suspended matter. Fill the sample in a clean tube and compare its colour with the 22 mixtures of the Forel-Ule colour scale. Find the matching colour and note the colour (viz., blue, green, etc.) of sample from this scale.

3.1.3. Temperature

Impinging solar radiation and the atmospheric temperature bring about interesting spatial and temporal thermal changes in natural waters which manifest in setting up of convection currents and thermal stratification. Discharge of heated effluents also bring about thermal changes in natural waters (thermal pollution). Temperature is basically an important factor for its effects on chemical and biological reactions in water. A rise in temperature of water accelerates chemical reactions, reduces solubility of gases, amplifies taste and odour, and elevates metabolic activity of organisms.

Table 1. Forel-Ule colour scale.

Solution	I	II	III	IV	V	VI	VII	VIII	IX	X
A	100	98	95	91	86	80	73	65	56	46
B	0	2	5	9	14	20	27	35	44	54
C	0	0	0	0	0	0	0	0	0	0
Colour	Blue		Greenish blue		Bluish green			Green		

Solution	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII
A	35	35	35	35	35	35	35	35	35	35	35	35
B	65	60	55	50	45	40	35	30	25	20	15	10
C	0	5	10	15	20	25	30	35	40	45	50	55
Colour	Greenish yellow					Yellow			Brown			

1. Surface water temperature

Material
A mercury thermometer of 0 to 50 °C range and with 0.2 °C least count.

Method
Take surface water in a plastic container and record its temperature immediately by dipping the thermometer for about one minute.

2. Subsurface temperature

I. Using thermos-flask sampler

Material
A sampler made of thermos-flask and having an inbuilt thermometer (Fig.5).

Method
Lower the sampler in water to the desired depth and close it. Draw the sampler out of water and read the temperature.

II. Using reversing thermometer

Material
A reversing thermometer (Fig.7): It consists of a large reservoir of mercury connected with a distal bulb by means of a fine capillary. Just

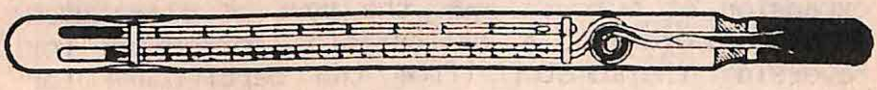

Fig.7. Reversing thermometer.

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above the reservoir this capillary is constricted and has a side branch at this point, above which it makes a loop and then becomes straight to join the bulb. The volume of murcury above the constriction depends upon the temperature and when the thermometer is reversed at 180° with the help of a messenger, the murcury thread breaks down at constriction and murcury fills the bulb and a part of capillary. An ordinary murcury thermometer, called auxillary thermometer, is attached on one side of reversing thermometer.

Method

Lower the reversing thermometer unit to the desired depth, wait for a few minutes and reverse the thermometer with the help of a messenger. Take out the unit and note the readings in two thermometers.

Calculation

$$T = T_1 + T'$$

$$T' = \frac{(T_1 - t)\,(T_1 - V_o)}{K - 100} + I$$

Where, T = corrected temperature of reversing thermometer (°C); T_1 = uncorrected reading of reversing thermometer (°C); t = temperature recorded from auxillary thermometer (°C); V_o = volume of small bulb and of capillary upto 0°C graduation; K = a constant (= 6100) depending upon the relative expansion of murcury and the type of glass used in thermometer; I = caliberation correction for reversing thermometer (from the certificate for individual thermometer).

III. Other methods

Other methods employed for subsurface temperature recording are the thermister, thermophone, and bathythermograph etc.

3.1.4. Annual heat budget

Annual heat budget of a lake is the amount of heat required to raise its water from minimum winter temperature to maximum summer temperature.

Material

i. Temperature recording device, like thermometer etc.; and ii. depth gauge, like weight tide to a graduated rope.

Method

Record, round the year, the temperature at various strata of body of water, and its depth. From these data calculate the mean summer temperature, mean winter temperature, and mean depth. Calculate annual heat budget of tropical and subtropical waters as follows.

Calculation

$$AHB = Z^{S}(T^{S}_{m} - T^{W}_{m}) + (Z^{W} - Z^{S}) \times 470$$

Where, AHB = annual heat budget (cal/ cm^2/yr); Z^S = mean depth during summer (cm); Z^W = mean depth during winter (cm); T^S_m = mean temperature during summer (°C); and T^W_m = mean temperature during winter (°C).

470 is the factor computed from latent heat of vaporisation (i.e., 540 cal) minus work done against external pressure (i.e.,approx. 70 cal).

Mean temperature (during summer or winter) is calculated as follows:

$$T_m = \frac{T_1 + T_2}{2}(P_1) + \frac{T_2 + T_3}{2}(P_2) + \ldots + \frac{T_n - 1 + T_n}{2}(P_n)$$

Where, T_m = mean temperature ; T_1, T_2, T_3 = temperatures at respective levels bounding the various strata of water from surface to downward; T_n = temperature at lowermost level of last stratum; P_1 , P_2 , P_n ⁄= percentage of entire lake volume which the first, second, and the last strata respectively constitute (e.g., if one stratum constitutes 10% of the entire lake volume, then the P = 10/100 = 0.1).

3.1.5. Transparency (Light penetration)

Solar radiation is the major source of light energy in natural waters in that it gets transformed into potential energy by biochemical reactions. Thus governing the primary productivity of aquatic systems it indirectly controls the whole biological set-up inside the body of water. The depth to which the light attenuation is 1% of what received on surface is called **compensation level** where photosynthesis of the system is considered to be equal to respiration. Zone above compensation level is the **trophogenic zone** where photosynthesis is in excess of respiration, while the zone below this is the **tropholytic zone** where respiration is in excess of photosynthesis.

Transparency of water is inversely proportional to the turbidity, which in turn is directly proportional to the amount of suspended organic and inorganic matter.

I. Secchi disc method

Material

Secchi disc (Fig.8): It was deviced by A.Secchi (1865), an Italian scientist. It is a metallic disc of 20 cm diameter with four quadrants on upper surface painted black and white alternately. It has a hook in the centre of upper part, to which a graduated cord is

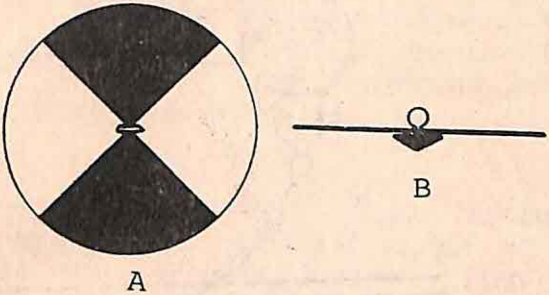

Fig.8. Secchi disc. A. top view, B. side view.

tied. On lower surface it has a centrally placed weight which facilitates the sinking of the disc in proper position.

Method

Lower the Secchi disc in water and note the depth (cm) at which it disappears. Now raise the disc slowly and note the depth at which it reappears.

Calculation

$$\text{Secchi disc transparency (cm)} = \frac{A + B}{2}$$

Where, A = depth at which Secchi disc disappears (cm); and B = depth at which Secchi disc reappears (cm).

$$\text{Euphotic limit (cm)} = \text{SDT} \times 2.5$$

$$\text{Vertical attenuation coefficient} = \frac{1.9}{\text{SDT}}$$

Where, SDT = Secchi disc transapency (cm).

II. Other methods

Modern underwater photometer (Fig.9) is the conven-

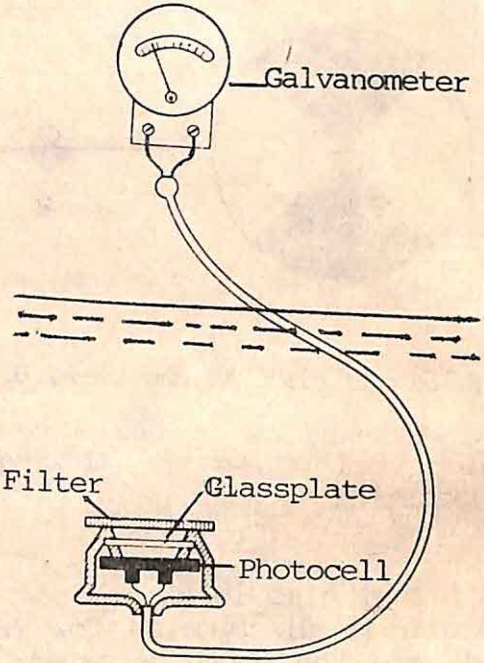

Fig.9. Underwater photometer.

ient and accurate decive to measure the light intensity at different depths of water. The intensity is read in lux.

3.1.6. Turidity

Turbidity in natural waters is caused by suspended matter like clay, silt, organic matter, phytoplankton, and other microscopic organisms. It is actually the expression of optical property (Tyndall effect) in which the light is scattered by the suspended particles present in water. Scattering of light is dependent upon the size, shape, and refractive index of such particles.

Turbidity, when largely because of phytoplankton, is considered as an index of productivity, but on the contrary, when because of suspended matter other than phytoplankton, it restricts the light penetration in water resulting in reduced primary production (photosynthesis).

Material

i. Nephelometer (Turbidimeter): This instrument is used to measure the scattered light at the right angle of the path of incident light.
ii. Sample tubes: These should be made of colour and scratch-free glass.
iii. Standard turbidity suspension: Dissolve 1 g of hydrazine sulphate in distilled water to prepare 100 ml of solution (a). Dissolve 10 g of hexamethylene tetramine in distilled water to prepare 100 ml of solution (b). Mix 5 ml of each of the solutions (a and b) in a 100 ml volumetric flask and allow to stand for 24 hours at about 25 °C. Dilute it with distilled water to 100 ml mark. This is a suspension having 400 NTU (Nephelometric Turbidity Unit), and can be stored for about a month.
Dilute 10 ml of above stock solution (of 400 NTU) to 100 ml with distilled water. This standard solution has 40 NTU. This can be stored for about a week.

Method

Set the nephelometer at 100 using 40 NTU standard suspension. In doing so, every per cent on the scale will be equal to 0.4 NTU turbidity. Shake the sample thoroughly and let the air bubbles subside. Thake the sample in nephelometer sample tube and find out the value on scale. If the sample has turbidity more than 40 NTU, dilute it so that its turbidity can be read on the same scale.

Calculation

Turbidity (NTU) = Nephelometer reading x 0.4 x dilution factor

3.2. CHEMICAL PARAMETERS

3.2.1. Hydrogen ion concentration (pH or Potentia hydrogenii)

pH is a scale of intensity of acidity or alkalinity and measures the concentration of hydrogen ions in water. If free H^+ are more than OH^- ions, the water will be acidic, or alkaline the other way round. The most chemically pure water is partly dissociated, and a temperature of 22 °C 10^{-14} g molecules of water per litre is equally dissociated into H^+ and OH^- ions. The concentration of hydrogen ions thus being 10^{-7}. Instead of the inconvenient number 10^{-7} the pH value is expressed as the negative logarithm of the hydrogen ion concentration. Thus pH 7 indicates neutral water, pH 7 to 14 alkaline, and pH below 7 acidic.

pH of natural waters varies around 7, generally over 7 (i.e., alkaline) due to presence of sufficient quantities of carbonates. It increases during day largely due to photosynthetic activity (consumption of carbon dioxide), whereas decreases at night due to respiratory activity. Factors like exposure to air, temperature, and disposal of industrial wastes etc. also bring about changes in pH.

Reserve pH (RpH) is that value obtained by a second pH determination made after the sample has been thoroughly aerated. Free carbon dioxide in the sample is partly responsible for the initial pH reading; aeration causes the release of the free carbon dioxide and measurement after aeration shows a RpH value higher on the alkaline side of pH scale.

I. Electrometric method (Using pH meter)

Material

i. pH meter with glass and calomel electrodes or a combind electrode. Prefer portable field pH meter which has a single electrode and is convenient to use.

ii. Thermometer.

iii. Buffer solutions, preferably of pH 4.0 and 9.2. Buffers of different pH can be prepared by dissolving standard pH tablets in 100 ml of distilled water, or as follows:

a. Phthalate buffer: Dissolve 10.2 g of potassium hydrogenphthalate in distilled water to make 1 l of buffer.

b. Phosphate buffer: Dissolve 3.40 g of KH_2PO_4 and 4.45 g of $Na_2HPO_4.2H_2O$ in distilled water to make 1 l of buffer.

c. Borax buffer: Dissolve 3.81 g of $Na_2B_4O_7.10H_2O$ in distilled water to make 1 l of buffer.

The pH of these buffers at various temperatures shown in Table 2.

Table 2. The pH value of three buffers at different temperatures.

Temperature °C	Phthalate buffer	Phosphate buffer	Borax buffer
0	4.01	6.98	9.46
5	4.00	6.95	9.39
10	4.00	6.92	9.33
15	4.00	6.90	9.27
20	4.00	6.88	9.22
25	4.01	6.86	9.18
30	4.01	6.85	9.14

Method

Read carefully the operation manual of pH meter to be used. Put the switch on and see that the meter reads pH 7. Wash the electrode with distilled water and connect it with the pH meter. Dip the electrode in buffer of pH 4.0 and move the temperature compensation knob to the temperature of buffer. Turn selector switch to pH range of 0-7 and adjust the set buffer knob until the meter reads pH 4.0. Move selector switch to zero. Remove the electrode from buffer,wash it with distilled water, and dip in a buffer of pH 9.2. Turn the selector switch to pH range of 7 - 14 and adjust the set buffer knob until the meter reads pH 9.2. In doing so, the meter is calibrated both for pH ranges from 0 to 7 and from 7 to 14. Put selector switch to zero. Wash the electrode with distilled water and dip in the sample. Adjust temperature compensation knob to the temperature of sample. Put selector switch to pH range of 0 to 7 and read the meter for the pH of sample. If pH exceeds 7, move selector switch to pH range of 7 to 14 and read the meter. Turn the selector switch to zero, switch off the instrument, and remove the electrode. Keep the electrode dipped in distilled water when not in use.

II. **Colorimetric method** (Using pH indicator)

Material

i. BDH universal indicator; ii. Lovibond comparator; and iii. cuvettes.

Method

Take 5 ml of water sample in two cuvettes. Place one cuvette in the cavity of comparator facing colour disc. Add two drops of universal pH indicator to the other cuvette. Shake gently and place it in the other cavity of comparator box. Match the colour developed in the sample with the standard colour disc of comparator and record the corresponding pH.

3.2.2. Electrical conductivity (EC)

Pure water is a poor conductor of electricity. Acids, bases, and salts in water make it relatively good conductor of electricity. Such substances are called **electrolytes**; those with poor solubility in water are called weak electrolytes and those with high solubility the strong electrolytes. In other words, electrolytes in a solution dissociate into positive (cations) and negative (anions) ions and impart conductivity. Thus higher the concentration of electrolytes in water the more is its electrical conductance (i.e., lesser the resistance). It is claimed that, other things being equal, the richer a body of water in electrolytes the greater is its biological productivity.

Conductance is the reciprocal of the resistance involved and the unit of measure of conductance is **reciprocal ohm** designated as **mho** or **Siemens (S).** When electrical conductivity is measured between the electrodes having a surface area of 1 cm^2 and placed at a distance of 1 cm, the unit of conductivity is **S/cm.** Because conductivity varies with temperature, it is conveniently reported at 25 °C.

The conductance of distilled water ranges from 1 to 5 umho (or µS). Waters having up to 20 mmho (or mS) conductance are considered to be suitable for irrigation.

Material
i. Conductivity meter; ii.conductivity cell; and iii. thermometer.

Method
Study carefully the operation manual

of the conductivity meter. Note the temperature of sample and adjust the temperature compensation knob of the conductivity meter to the temperature of sample. Keep the selector switch to x1000 and calibrate to CAL mark. Dip the conductivity cell in the sample contained in a beaker and connect the cell terminals to the sockets provided in the instrument. If meter shows negligible deflection, disconnect the cell terminals. Move the selector switch to x100 and calibrate to CAL mark. Reconnect the cell terminals and note the deflection. If it is still negligible, disconnect the cell, move the selector switch to x10 and calibrate to CAL mark. Reconnect the cell and note the deflection (dial reading). Switch off the meter, disconnect the cell and wash it with distilled water.

Calculation

$$\text{EC (S)} = \text{DR} \times \text{SS}$$

Where, EC = electrical conductivity; DR = dial reading; and SS = value of selector switch.

In meters not provided with selector switch and temperature compensation knob, EC is computed as follows:

$$\text{EC (S)} = \text{OEC} \times \text{CC} \times \text{TF at } 25\ ^{\circ}\text{C}$$

Where, OEC = observed conductance; CC = cell constant (supplied by the manufacturer); and TF = temperature factor as may be obtained from Table 3.

3.2.3. Redox Potential

Redox potential is the measure of oxidating or reducing power of the water. In the process of oxidation a substance loses electrons while

Table 3. Factors for converting the conductivity value at 25 °C.

°C	Factor	°C	Factor	°C	Factor
3	1.62	13	1.27	23	1.04
4	1.58	14	1.24	24	1.02
5	1.54	15	1.21	25	1.00
6	1.50	16	1.19	26	0.98
7	1.46	17	1.16	27	0.97
8	1.42	18	1.14	28	0.95
9	1.39	19	1.12	29	0.93
10	1.36	20	1.10	30	0.92
11	1.33	21	1.08	31	0.90
12	1.30	22	1.06	32	0.89

in reduction it gains electrons. As such, in a system processes of oxidation and reduction take place simultaneously. When a platinum electrode is immersed in such system, a potential, depending upon the ratio of oxidized and reduced states, is developed and is called as redox potential.

The value of redox potential gives a picture of oxidation and reduction processes going on in water; a low value of redox potential indicates high reduction while a high value of it indicates high oxidation. For better aerobic treatment of waste water redox potential should be high (+200 to +600 mV) while for anaerobic treatment it should be low (-100 ro -200 mV).

Material

i. Milliamperemeter or a pH meter with facility to measure potential in millivolts, ii. platinum electrode; and iii. calomel electrode.

Method
Dip the two electrodes (platinum and calomel) together in water sample contained in a beaker, and connect them with milliamperemeter. Switch on the meter and turn selector switch to 0 to +1000 mV range. Record the deflection on dial. If it is beyond the scale, turn selector switch to 0 to -1000 mV scale and record the deflection. Switch off the meter. Remove and wash the electrodes. Platinum electrode is usually cleaned by heating it in 50% sulphuric acid and leaving it in same for about 12 hours.

When a pH meter with the facility to measure potential is to be used, replace the glass electrode of pH meter with platinum electrode. Set the selector switch to mV. Immerse the electrode in sample and read the potential on millivolt scale.

3.2.4. Total dissolved solids

A large number of salts are found dissolved in natural waters, the common ones are carbonates, bicarbonates, chlorides, sulphates, phosphates, and nitrates of calcium, magnesium, sodium, potassium, iron, and manganese etc. A high content of dissolved solids elevates the density of water, influences osmoregulation of freshwater organisms, reduces solubility of gases (like oxygen) and utility of water for drinking, irrigational, and industrial purposes. It is especially an important parameter in the analysis of saline lake, coastal, esturarine, and marine waters. This factor, having high value in such waters, is often expressed as g/l or ppt (parts per thousand) instead of mg/l or ppm (parts per million).

Material
i. Evaporating dish; ii. chemical balance, iii. desiccator; iv. hot water bath; and filter paper

(Whatman, No. 4).

Method

Take an evaporating dish of suitable size, make it clean and dry, and weigh it. Filter 250 to 500 ml of sample through a filter paper (Whatman No.4) and take the filterate in evaporating dish. Evaporate the sample on hot water bath. When whole water is evaporated note the weight of evaporating dish after cooling it in a desiccator.

Calculation

$$\text{TDS (g/l)} = \left(\frac{A - B}{V} \right) \times 1000$$

Where, TDS = total dissolved solids; A = final weight of evaporating dish (g); B = initial weight of evaporating dish (g); and V = volume of sample taken (ml).

If total solids, instead of total dissolved solids, are to be computed, do not filter the sample.

3.2.5. Dissolved oxygen

Oxygen dissolved in water, often referred to as DO, is a very important parameter of water quality and is an index of physical and biological processes going on in water. There are two main sources of dissolved oxygen in water, i. diffusion from air, and ii. photosynthetic activity within water. Diffusion of oxygen from air to water is a physical phenomenon and depends upon solubility of oxygen which, in turn, is influenced by factors like temperature, water movements, and salinity etc. Photosynthetic activity is a biological phenomenon carried out by autotrophs (mainly phytoplankton in water) and depends upon autotroph population, light conditions, and available gases etc.

Non-polluted surface waters are normally saturated with dissolved oxygen, while presence of oxygen demanding pollutants (like organic wastes) causes rapid depletion of dissolved oxygen from water. Oxydizable inorganic substances, like hydrogen sulphide, ammonia, nitrites, ferrous iron etc. also cause decrease in dissolved oxygen. Eutrophic waters have a wide range of dissolved oxygen content while oligotrophic ones have a narrow range. Eutrophic bodies of water show a clinograde oxygen curve in which dissolved oxygen is more at surface and depletes fast with the depth, while oligotrophic bodies of water show an orthograde oxygen curve in which dissolved oxygen increases with the depth.

Oxygen is considered to be an A-1 limiting factor, especially in lakes and in waters with a heavy load of organic material. Organisms have specific oxygen requirements, for example, 2-5 mg/l for most of the fishes. Low dissolved oxygen may prove lethal for many of the organisms.

I. Winkler's method

Material

i. BOD bottles (100 - 300 ml); ii. laboratory glassware like pipettes, burette, flasks, beakers, etc. and iii. reagents, as given below:

A. Sodium thiosulphate solution (0.025 N): Dissolve 6.205 g of sodium thiosulphate in previously boiled distilled water and make up the volume to 1 l. Add a pallet of sodium hydroxide as a preservative. Keep the solution in coloured bottle.

B. Manganous sulphate solution: Dissolve 100 g of manganous sulphate in 200 ml of previously boiled distilled water and filter the solution.

C. Alkaline potassium iodide solution: Dissolve 100 g of potassium hydroxide and 50 g of potassium iodide in 200 ml of previously boiled distilled water.

D. Starch indicator: Dissolve 1 g of starch in 100 ml of warm distilled water and add a few drops of toluene or formaldehyde as preservative.
E. Sulphuric acid: Concentrated; sp.gr. 1.84; 18 M.

Method

Take a glass stoppered BOD bottle of known volume (100 - 300 ml) and fill it with sample avoiding any bubbling. No air should be trapped in bottle after the stopper is placed. Open the bottle and pour in it 1 ml of each manganous sulphate (reagent B) and alkaline potassium iodide (reagent C) solutions using separate pipettes. If the volume of sample is over 200 ml, add 2 ml of each reagent instead of 1 ml. A precipitate will appear. Place the stopper and shake the bottle thoroughly. Sample at this stage can be stored for a few days, if required. Add 2 ml of sulphuric acid (reagent E) and shake thoroughly to dissolve the precipitate. Transfer gently (avoiding bubbling) whole content, or a known part of it, in a conical flask. Put a few drops of starch indicator (reagent D). Titrate against sodium thiosulphate solution (reagent A) and note the end point when initiial blue colour turns to colourless.

Calculation

If whole content is used for titration:

$$\text{DO (mg/l)} = \frac{V_1 \times N \times 8 \times 1000}{V_2 - V_3}$$

If a fraction of the contents is used for titration:

$$\text{DO (mg/l)} = \frac{V_1 \times N \times 8 \times 1000}{V_4 \left(\frac{V_2 - V_3}{V_2} \right)}$$

Where, DO = dissolved oxygen; V_1 = volume of titrant (ml); N = normality of titrant (0.025);

V_2 = volume of sampling bottle after placing the stopper (ml); V_3 = volume of manganous sulphate + potassium iodide solutions added (ml); and V_4 = volume of fraction of the contents used for titration (ml).

To obtain the value of DO in ml/l divide the DO in mg/l by 1.43.

II. Modified Winkler's method

The original Winkler's method is modified so that the interferance due to organic matter and chlorides present in sample is avoided by using sodium azide in alkaline potassium iodide solution.

Material

Same as those for original Winkler's method except that the alkaline potassium iodide solution is prepared as given below:

Dissolve separately 350 g of KOH and 75 g of KI in distilled water, mix the two and make the volume 500 ml with distilled water. Dissolve separately 5 g of sodium azide (NaN_3) in 20 ml of distilled water. Mix alkaline iodide and sodium azide solutions.

Method

Same as that for original Winkler's method.

Calculation

Same as that for original Winkler's method.

III. Oxygen meter method

This method is convenient, quick, and reasonably accurate.

Material

i. Oxygen meter with DO probe; ii. electrical stirrer; and iii. 5% sodium sulphite solution.

Method
Read the operation manual of the instrument carefully and adjust it accordingly. Stirr constantly the 5% sodium sulphite solution and dip in it the DO probe. Set the meter to zero mark. Now dip the DO probe in water sample being constantly stirred, and record the dissolved oxygen in mg/l from the dial.

3.2.6. Per cent oxygen saturation

The solubility of oxygen (like other gases) in water depends largely upon temperature and atmospheric pressure. Amount of oxygen that can be dissolved in water at particular temperature and pressure is fixed and such water is said to be 100 per cent saturated with oxygen. Processes like high photosynthetic activity may cause exceptionally high concentration of oxygen and thus its supersaturation in water; while high respiration and salinity may lead to oxygen in water below saturation level.

Method
The per cent oxygen saturation in water can be determine using nomogram shown in Fig.10. This method is applicable for surface layers of water only as at deeper layers hydrostatic pressure plays important role in the relationship.

Connect temperature and pressure values and mark the intersection (e.g., a and b in the Fig.10) on line R. Now connect the intersection at line R with dissolved oxygen value (mg/l) and extrapolate to connect with per cent saturation scale. Read the value as per cent dissolved oxygen saturation.

3.2.7. Free carbon dioxide

Rain water according to its solution equilibrium

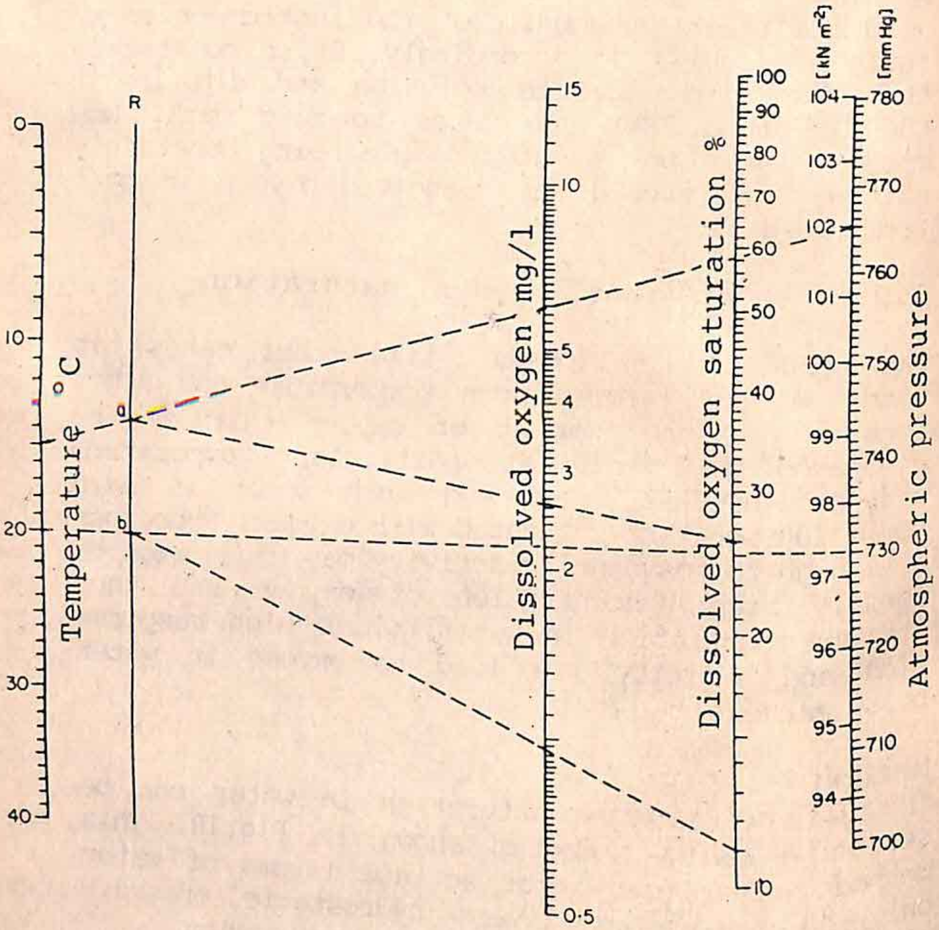

Fig. 10. Nonogram for computing % oxygen saturation.

with the atmospheric air and the absorption coefficient of water for carbon dioxide, contains about 0.6 mg CO_2 per litre. When precipitated water percolates through the soil, additional CO_2 is dissolved out of soil air. Groundwaters are, therefore, extra rich in CO_2. Respiratory activity of aquatic organisms and the process of decomposition are important sources of CO_2 in bodies of surface waters. Free CO_2 combines with water partly to form carbonic acids (H_2CO_3), as such in normal practice free CO_2 is distinguished as CO_2 and H_2CO_3 .

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

Water containing free CO_2 reacts with limestone or chalk ($CaCO_3$) of soil or sediment, producing calcium bicarbonate [$Ca(HCO_3)_2$]. If more CO_2 is present in water than is in equilibrium with air, much more $CaCO_3$ will be dissolved (i.e. convrted into calcium bicarbonate) because this extra CO_2 keeps the pH low. As a result, concentration of carbonate ($CO_3^=$) ions will reduce and that of bicarbonate (HCO_3^-)ions will increase. Calcium bicarbonate remains in solution if it is in equilibrium with a certain amount of free CO_2 in water. If the excess CO_2 is removed from water, the bicarbonate is decomposed into calcium carbonate and free CO_2 . Because CO_2 is produced by this process it continues so long as sufficient equilibrium CO_2 is present in water.

$$\underset{\text{(weakly soluble)}}{CaCO_3} + H_2O + CO_2 \rightleftharpoons \underset{\text{(readily soluble)}}{Ca(HCO_3)_2}$$

This reaction will continue until the equilibrium between HCO_3^- , $CO_3^=$, and CO_2 is established.

At varying pH, different proportions of these species of carbon dioxide (free CO_2, $CO_3^=$, and HCO_3^-) present in CO_2-H_2O system are shown in Fig. 11. At pH beyond 8.3 because the concentration of free CO_2 is negligible, the bicarbonates begin to decompose and precipitate as carbonates. Between pH 0.0 and 6.35 almost all the species of CO_2 are present in the form of carbonic acid; between pH 6.35 and 10.33 in the form of HCO_3^-; and between pH 10.33 and 14.0 in the form of $CO_3^=$. .

Free CO_2 dissolved in water is the only source

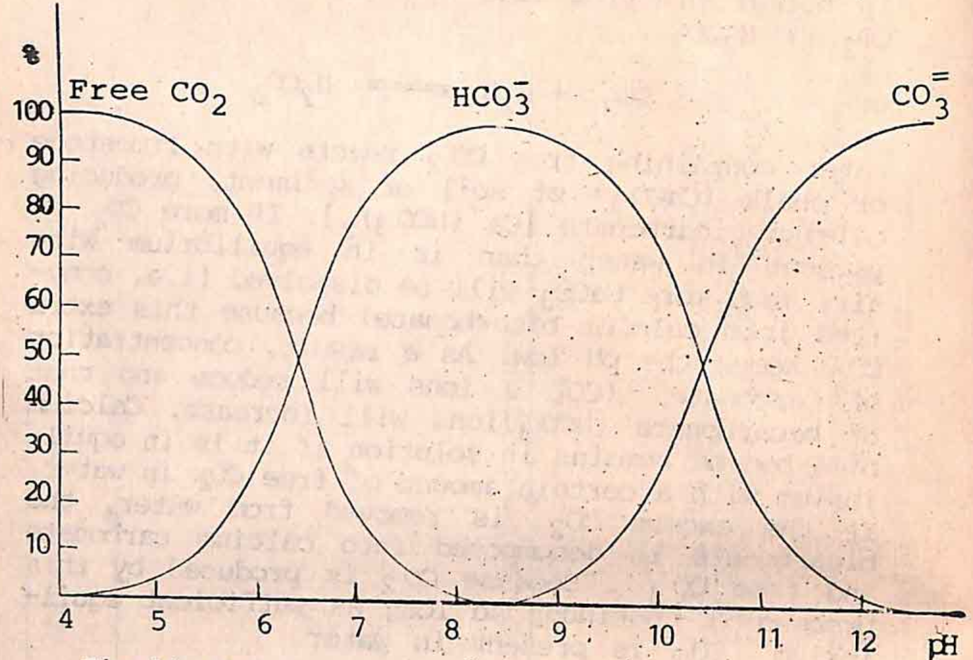

Fig.11. Relationship between pH and percentage of carbon dioxide (free CO_2, HCO_3^-, and $CO_3^=$).

of carbon that can be used in photosynthetic activity of aquatic autotrpohs. Once fixed by autotrophs it can further be utilized by organisms at other trophic levels. In the absence of free CO_2 , the bicarbonates are converted into carbonates releasing CO_2 which is utilized by autotrophs, thus making the water more alkaline.

Material

i. Laboratory glassware; and ii. reagents, as given below:

A. Sodium hydroxide solution (0.2272 N): Dissolve 0.909 g of sodium hydroxide in CO_2 free (boiled and cooled) distilled water and make the volume 1 l. Standardize the solution.

B. Phenolphthalein indicator: Dissolve 1 g of phenolphthalein in 100 ml of ethyl alcohol and add 100 ml of distilled water. Add NaOH solution (reagent A) drop by drop until a faint pink colour appears.

Method

After collection analyse the sample as soon as possible. Take 50 ml of sample in a flask and add 2-3 drops of phenolphthalein indicator (reagent B). If the colour turns pink, free CO_2 is absent in sample. If the sample remains colourless, titrate it against sodium hydroxide solutior (reagent A) until pink colour appears (end point).

Calculation

$$\text{Free } CO_2 \text{ (mg/l or ppm)} = \frac{Vt \times 1000}{Vs}$$

Where, Vt = volume of titrant (ml); and Vs = volume of sample (ml).

3.2.8. Alkalinity

(Carbonate, Bicarbonate, Hydroxide, and Total alkalinity)

Alkalinity of water is its capacity to neutralize a strong acid and is characterized by the presence of hydroxyl (OH^-) ions capable of combining with hydrogen (H^+) ions. A number of bases viz., carbonates, bicarbonates, hydroxides, phosphates, nitrates, silicates, borates etc. contribute to the alkalinity, however, in natural waters carbonates, bicarbonates, and hydroxides are considered to be the predominant bases. Thus alkalinity may be expressed as total alkalinity or alakainity due to individual bases.

In natural waters most of the alkalinity is caused due to CO_2 . The free CO_2 combines with water partly to form carbonic acid (H_2CO_3), which is further dissociated into hydrogen (H^+) and bicarbonate (HCO_3^-) ions. The HCO_3^- ions thus formed get further dissociated into H^+ and carbonate ($CO_3^=$) ions.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

$$HCO_3^- \rightleftharpoons CO_3^= + H^+$$

Carbonate and bicarbonate ions in water further yield hydroxyl(OH^-) ions, carbonates produce double the OH^- ions than what produced by bicarbonates. This leads to the rise in pH.

$$CO_3^= + 2H_2O \rightleftharpoons H_2CO_3 + 2OH^-$$

$$HCO_3^- + H_2O \rightleftharpoons H_2CO_3 + OH^-$$

Besides this, action of water containing free CO_2 on limestone also causes alkalinity as shown below:

$$\underset{\text{(weakly soluble)}}{CaCO_3} + H_2O + CO_2 \rightleftharpoons \underset{\text{(readily soluble)}}{Ca(HCO_3)_2}$$

Natural waters with high alkalinity are generally rich in phytoplankton, especially the blue-greens. In highly productive waters the alkalinity ought to be over 100 mg/l.

Hard water lakes are characterized by negative values of free CO_2 due to the withdrawal of bicarbonates at a greater rate than carbonates precipitated, and by pH ranging from about 8.5 upwards. Carbonate content amounts to over 40 mg/l, often reaching 200 mg/l or more, which may precipitate as marl.

Material

i. Laboratory glassware; and ii. reagents, as given below:

A. Sulphuric acid (0.02N): Dilute 2.8 ml of concentrated sulphuric acid to 1 l using distilled water. Dilute 200 ml of this stock solution (0.1N) to 1 l using distilled water to prepare 0.02N sulphuric acid titrant. Standardized the solution.

B. Phenolphthalein indicator: Same as that used in free CO_2 estimation (see 3.2.7).

C. Methyl orange indicator: Dissolve 0.1 g of methyl orange in 200 ml of distilled water.

Method

After collection analyse the sample as soon as possible. Take 50 ml of sample in a flask and add 2-3 drops of phenolphthalein indicator (reagent B). If a slight pink colour appears, phenolphthalein alkalinity (due to hydroxide or carbonate) is present (non-appearance of pink colour is indicative of presence of free CO_2 but absence of phenolphthalein alkalinity). Titrate the solution against sulphuric acid (reagent A) until solution becomes colourless (end point). Note the reading as **p** . Add 2-3 drops of methyl orange indicator (reagent C) in the same flask and continue to titrate against sulphuric acid (reagent A) until yellow colour of solution turns orange (end point). Note the reading as **t** which is the volume of titrant used for both the titrations.

Calculation

$$\text{Phenolphthalein alkalinity (as } CaCO_3\text{, mg/l)} = \frac{p \times 1000}{S}$$

$$\text{Total alkalinity (as } CaCO_3\text{, mg/l)} = \frac{t \times 1000}{S}$$

Where, p = volume of titrant used against phenolphthalein indicator (ml); S = volume of sample (ml); and t = total volume of titrant used for the two titrations (ml).

The value of different forms of alkalinities (hydroxide, carbonate, and bicarbonate) in terms of $CaCO_3$ (mg/l) can be computed using Table 4.

Table 4. Value of hydroxide, carbonate, and bicarbonate alkalinities. P = Phenolphthalein alkalinity; T = Total alkalinity.

Results	Value of alkalinity expressed in $CaCO_3$		
	Hydroxide	Carbonate	Bicarbonate
$P = 0$	0	0	T
$P < \frac{1}{2} T$	0	2P	T-2P
$P = \frac{1}{2}T$	0	2P	0
$P > \frac{1}{2}T$	2P-T	2(T-P)	0
$P = T$	T	0	0

To compute the concentration of OH^-, $CO_3^=$, and HCO_3 ions following calculations are employed:

$$OH^- (mg/l) = \text{Hydroxide alkalinity} \times 0.34 \text{ (in } CaCO_3\text{, mg/l)}$$

$$CO_3^= (mg/l) = \text{Carbonate alkalinity} \times 0.60 \text{ (in } CaCO_3\text{, mg/l)}$$

$$HCO_3^- (mg/l) = \text{Bicarbonate alkalinity} \times 1.22 \text{ (in } CaCO_3\text{, mg/l)}$$

3.2.9. Total carbon dioxide

Total carbon dioxide is the sum of all the three species of CO_2 (i.e. free CO_2, $CO_3^{=}$, and HCO_3^{-}) present in water. It may be used as an index of trophic status.

Method

Estimate the free CO_2, carbonate alkalinity, and bicarbonate alkalinity as explained earlier.

Calculation

$$\text{Total } CO_2(mg/l) = \text{Free } CO_2(mg/l) + 0.88\ (B+C)$$

Where, B = bicarbonate alkalinity (in $CaCO_3$,mg/l); and C = carbonate alkalinity (in $CaCO_3$,mg/l)/2.

3.2.10. Chloride

Inland natural waters, in general, have low chloride concentration, often less than that of bicarbonates and sulphates. However, inland saline waters, coastal, estuarine, and seawater are characterized by moderate to very high chloride content. In natural fresh waters high concentration of chlorides is considered to be an indicator of pollution due to organic wastes of animal origin (animal excreta has high quantity of chlorides alongwith nitrogenous wastes). Industrial effluents may increase the chloride content in natural waters. Chloride content above 250 mg/l makes a water salty in taste, however, a level upto 1000 mg/l is safe for human consumption.

Material

i. Laboratory glassware; and ii. reagents, as given below:

A. Silver nitrate solution (0.02N): Dissolve 3.397 g of silver nitrate in distilled water and dilute to 1 l. Store the solution in a dark glass bottle.

B. Potassium chromate indicator: Dissolve 10 g of potassium chromate in about 20 ml of distilled water. Add a few drops of 0.02N silver nitrate solution (reagent A) to produce a red precipitate. Let stand it for about 12 hours. Filter and dilute the filterate to 1 l with distilled water.

Method
Take 10 ml of sample in a flask and add 5-6 drops of potassium chromate indicator (reagent B). The colour of sample becomes yellow. Titrate against silver nitrate solution (reagent A) until a persistant brick red colour appears (end point).

Calculation

$$\text{Chloride (mg/l)} = \frac{V \times N \times 35.457 \times 1000}{S}$$

Where, V = volume of titrate (ml); N = normality of titrant (0.02); and S = volume of sample (ml).

The salinity of water, on the basis of its empirical relationship with chloride content, may be calculated as follows:

$$\text{Salinity (g/l)} = 0.03 + 1.805(\text{Chloride in mg/l})$$

3.2.11. Fluoride

Fluorides are more common in ground waters than in surface waters. The main sources of fluoride in water are different fluoride bearing rocks. The maximum permissible limit of fluoride in drinking water is recommended to be 1.5 mg/l by WHO. When present in high concentration it causes mottling of teeth, skeletal fluorosis, forward bending of vertebral column, deformation of knee joints and other parts of body, and even paralysis (paraplegia, quadriplegia).

Material
i. Spectrophotometer; ii. laboratory glassware; and iii. reagents, as given below:

A. Alizarin red solution: Dissolve 0.75 g of 3-alizarin sulphuric acid sodium salt (alizarin red S) in distilled water to prepare 1 l of solution.
B. Zirconyl acid solution: Dissolve 0.354 g of zirconyl chloride octahydrate ($ZrOCl_2.8H_2O$) in 600 ml of distilled water. Add slowly 33.3 ml of sulphuric acid (concentrated)followed by 101 ml of hydrochloric acid (concentrated). Cool and further add distilled water to make the volume 1 l.
C. Standard fluoride solutions: Dissolve 0.221 g of sodium fluoride in distilled water to make the volume 1 l. Take 100 ml of this sloution and dilute to 1 l by adding distilled water; this stock solution contains 10 mg F/l. Prepare a series of standard fluoride solutions, preferably in the range of 0.0 to 5.0 mg F/l at intervals of 0.5 mg F/l, by dilution of the stock solution with distilled water.

Method
Take 100 ml of sample in a flask and add 5 ml each of alizarin red solution (reagent A) and zirconyl acid solution (reagent B). Wait for 1 hour and then note the absorbance (S) on spectrophotometer at 520 nm. Use distilled water as blank.

Run the standard fluoride solutions of various concentrations (reagent C) in similar manner and record absorbance for each. Plot a standard curve between concentrations and absorbances of standard solutions (Fig.12). Find out the fluoride content of the sample by comparing its abbsorbance (S) with standard curve and express the result as mg F/l.

3.2.12. Sulphate

Sulphates are found in appreciable quantity in all natural waters, particularly high in arid and semi-arid reqions where natural waters in general

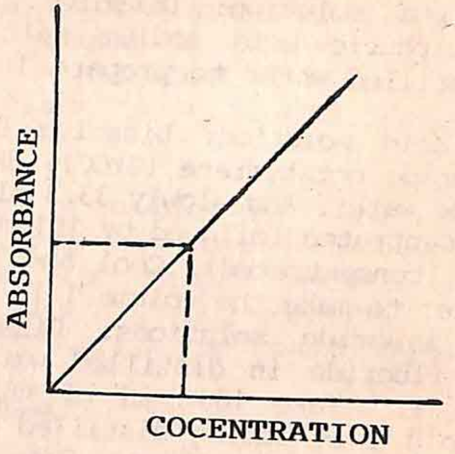

Fig.12. Standard curve between absorbance and concentration.

have high salt content. Domestic sewage and industrial effluents, besides biological oxidation of reduced sulphur species, may add to sulphate content of water. In a region where atmospheric sulphur content is high because of industrial and automobile emission, the rain water has high sulphur content.

Sulphate salts are mostly soluble in water and impart hardness. Waters with about 500 mg/l sulphate have a bitter taste and those with 1000 mg/l or more sulphate may cause intestinal disorders.

I. Turbidimetric method

This is the most routine and lesser time consuming method of sulphate estimation and is based on the precipitation of sulphate after adding barium.

Material

i. Laboratory glassware; ii. spectrophotometer; iii. magnetic stirrer; iv. filter paper

(Whatman No.1);and v. reagents, as given below:

A. NaCl-HCl solution: Dissolve 240 g of sodium chloride in a little distilled water. Add 20 ml of hydrochoric acid to it and dilute with more of distilled water to make the volume 1 l.

B. Glycerol-ethanol solution: Add 50 ml of glycerol to 100 ml of ethyl alcohol and shake well.

C. Barium chloride: Dry crystals.

D. Standard sulphate solutions: Dissolve 0.1479 g of anhydrous sodium sulphate in distilled water to make the volume 1 l. This solution contains 100 mg sulphate/l. Prepare standards of various strengths (preferably from 0.0 to 40.00 mg/l at the intervals of 5 mg/l) by diluting this stock solution.

Method

Filter the sample through filter paper (Whatman No.1) and take 50 ml of filterate in an Erlenmeyer flask. Add 10 ml of NaCl-HCl solution (reagent A), 10 ml of glycerol-ethanol solution (reagent B), and 0.15 g of barium chloride (reagent C). Stirr the sample with the help of a magnetic stirrer for about an hour. Measure the absorbance(S) against a distilled water blank at 420 nm using spectrophotometer.

Process the standard solutions of different strengths (reagent D) in similar way and record the absorbance for each. Plot a standard graph from these values putting strengths (mg/l) on one axis and absorbances on the other.

Deduce the sulphate content of the sample in mg/l by comparing the absorbance of sample(S) with standard curve.

II. Gravimetric method

This method can be very accurate, but is then time consuming. Moreover, if the sample is rich in interfering substances, like silica, the results cannot be accurate and this situation requires removal of interfering substances before processing the sample for sulphate estimation.

Material

i. Laboratory glassware; ii. crucible; iii. filter paper (Whatman No.1 & 42); and iv. reagents, as given below:

A. Methyl red indicator: Dissolve 50 mg of methyl red sodium salt in distilled water to prepare 50 ml of the indicator.

B. Barium chloride solution: Dissolve 100 g of barium chloride in distilled water to prepare 1 l of solution. Filter the reagent through filter paper (Whatman No.1).

C. Silver nitrate solution: Add 0.5 ml of concentrated nitric acid to 8.5 g of silver nitrate and dissolve it in distilled water to make the volume 500 ml.

D. Hydrochloric acid (50%): Dilute concentrated hydrochloric acid with distilled water in 1:1 ratio.

Method

If the sample contains low silica ($<$ 25 mg/l) it requires no removal of silica. Take 100 ml of sample in an Erlenmeyer flask and add 2-3 drops of methyl red indicator (reagent A). Adjust the pH to about 5.0 by adding hydrochloric acid (reagent D) until the colour changes from red to orange. Add about 2 ml of hydrochloric acid in excess. Boil this solution and add warm barium chloride solution (reagent B) slowly until the precipitation is completed. Heat the solution in a water bath at 90 °C for 2 hours. Filter the precipitate through ashless filter paper (Whatman No.42). Wash the precipitate with warm distilled water until the filterate shows no traces of chloride which can be tested by adding drops of silver nitrate solution (reagent C) to the filterate. Absence of any white turbidity in filterate after addition of silver nitrate solution indicates absence of chlorides. Dry the filter paper with precipitate on it and then ignite in a crucible at 800 °C for 1 hour. Cool the crucible in a desiccator and weigh the precipitate (of barium sulphate).

Calculation

$$\text{Sulphate (mg/l)} = \frac{W \times 411.5}{V}$$

Where, W = weight of precipitate (mg); and V = volume of sample (ml).

3.2.13. Hydrogen sulphide

In ground and surface waters sulphides occur usually in low concentrations and are caused due to reduction of sulphates and organic sulphur. However, in waters rich in organic matter and having reducing conditions (i.e. low redox potential) the sulpide content is often remarkably high. This is due to the anaerobic respiration of microorganisms during which they use sulphate as electron acceptor to decompose organic matter and reduce sulphate into sulphide. Specialized microorganisms are largely responsible for the following transformations under reducing conditions: $SO_4 \rightarrow H_2S$ (anaerobic sulphate reduction), disulphovibrio bacteria; and organic $S \rightarrow H_2S$, anaerobic heterotrophic microorganisms. Under oxidizing conditions microorganisms bring about following transformations: $H_2S \rightarrow S \rightarrow SO_4$, colourless, green, and purple sulphur bacteria; $H_2S \rightarrow SO_4$ (aerobic sulphide oxidizers), thiobacilli bacteria; and organic $S \rightarrow SO_4$, aerobic heterotrophic microorganisms. Thus the presence of hydrogen sulphide (H_2S) may well be considered as an index of organic pollution and low redox potential. Its concentration in water is inversely proportional to that of oxygen.

Material

i. Laboratory glassware; and ii. reagents, as given below:

A. Cadmium chloride solution: Dissolve 2 g of cadmium chloride in 100 ml of distilled water.

B. Hydrochloric acid (4N): Dilute 33.3 ml concentrated hydrochloric acid (12N) with distilled water to make the volume 100 ml.

C. Starch indicator: Dissolve 1 g of soluble starch in 100 ml of distilled water, heat and cool. If the indicator is to be stored, add a few drops of formaldehyde as preservative.

D. Sodium thiosulphate solution (0.025N): Dissolve 24.82 g of sodium thiosulphate in distilled water and make the volume 1 l (0.1N stock solution). Dilute 250 ml of this stock solution in a volumetric flask to 1 l with distilled water to prepare a 0.025N solution.

Method

Fill a glass stoppered bottle of known volume (about 100 ml) with sample. Avoide any bubbling while filling the bottle. Add 1 ml of cadmium chloride solution (reagent A), place the stopper and shake well the bottle. Let the bottle stand for over 24 hours to allow precipitate (of cadmium sulphide) to settle down. Decant the supernatant with the help of a pipette and dissolve the precipitate in 5 ml of hydrochloric acid (reagent B). Titrate the contents against sodium thiosulphate solution (reagent D) using starch indicator (reagent C). A change from dark blue colour to colourless solution is the end point. Run a distilled water blank in similar manner.

Calculation

$$H_2S\ (mg/l) = \frac{(A-B) \times N \times 17 \times 1000}{V}$$

Where, A = volume of titrant used against sample (ml); B = volume of titrant used against blank (ml); N = normality of titrant (0.025); and V = volume of sample (ml).

3.2.14. Total hardness

The total hardness of water is the sum of concentration of alkaline earth metal cations present in it. Calcium and magnesium are the principal cations imparting hardness, however, to a lesser extent cations like iron, manganese, and strontium are also responsible for it. Hardness when caused because of bicarbonates and carbonates of these cations is called **temporary hardness** since it can be removed by boiling the water. Sulphates and chlorides of these cations cause **permanent hardness** which is not removed by simple boiling of water. In general practice the hardness is measured as concentration of only calcium and magnesium (as $CaCO_3$) which are far high in concentration over other cations.

Hardness of water prevents lather formation with soap, therefore, hard water is not suitable for bathing and washing. Hard waters have high boiling point and so are not good for cooking too.

Material

i. Laboratory glassware; ii. reagents, as given below:

A. Ammonia buffer solution: Dissolve 13.5 g of ammonium chloride in 114 ml of concentrated ammonium hydroxide and add distilled water to make the volume 200 ml.

B. Erichrome black-T indicator: Dissolve 0.5 g of erichrome black-T dye in 100 ml of 80% ethyl alcohol.

C. EDTA solution (0.01 M): Dissolve 3.723 g of disodium salt of EDTA in distilled water to prepare 1 l of solution. Store in a polyethylene bottle.

Method

Take 50 ml of sample in an Erlenmeyer flask and add 1 ml of ammonia buffer solution (reagent A) and 4-5 drops of erichrome black-T indicator (reagent B). Titrate against EDTA solution (reagent C) until the wine red colour of solution turns blue (end point).

Calculation

$$\text{Total hardness (mg/l, as } CaCO_3\text{)} = \frac{T \times 1000}{V}$$

Where, T = volume of titrant (ml); and V = volume of sample (ml).

3.2.15. Calcium

Calcium is found in great abundance in all natural waters and its source lies in the rocks from which it is leached. Its concentration varies greatly in natural waters depending upon the nature of the basin.

Calcium is an important micronutrient in an aquatic environment. It is needed in especially large quantities by the molluscs and the vertebrates. Being an important contributor to hardness in water it reduces the utility of water for domestic use.

Material

i. Laboratory glassware; and ii. reagents, as given below:

A. Sodium hydroxide solution (8%): Dissolve 8 g of sodium hydroxide in distilled water to prepare 100 ml of solution.

B. Murexide indicator: Mix 0.2 g of ammonium purpurate and 100 g of sodium chloride and grind thoroughly.

C. EDTA solution (0.01 M): Dissolve 3.723 g of disodium salt of EDTA in distilled water to prepare

1 l of solution.

Method

Take 50 ml of the sample in an Erlenmeyer flask and add 1 ml of sodium hydroxide solution (reagent A) and a pinch of murexide indicator (reagent B). Titrate against EDTA solution (reagent C) until the pink colour turns purple (end point).

Calculation

$$\text{Calcium (mg/l)} = \frac{T \times 400.5 \times 1.05}{V}$$

Where, T = volume of titrant (ml); and V = volume of sample (ml).

To determine the calcium hardness to be expressed in mg/l as $CaCO_3$, employ following formula:

$$\text{Calcium hardness (mg/l, as } CaCO_3\text{)} = \frac{T \times 1000 \times 1.05}{V}$$

Where, T = volume of titrant (ml); and V = volume of sample (ml).

3.2.16. Magnesium

Like calcium, magnesium is also found in all natural waters and its source too lies in rocks. It is generally in low concentration than calcium.

Magnesium is a necessary constituent of chlorophyll without which no ecosystem could operate. Its high content reduces the utility of water for domestic use, while a concentration above 500 mg/l imparts water an unpleasant taste and renders it unfit for drinking purpose. High concentration of magnesium also proves to be diuretic and laxative.

Method & Calculation

Total hardness and calcium hardness of water as mg/l $CaCO_3$ are determined as discussed earlier (see 3.2.14, 3.2.15). From these values magnesium content is calculated as given below:

$$\text{Magnesium (mg/l)} = (T - C) \times 0.244$$

Where, T = total hardness (mg/l, as $CaCO_3$); and C = calcium hardness (mg/l, as $CaCO_3$).

3.2.17. Sodium

This cation occurs generally in lower concentration than calcium and magnesium in fresh waters, and makes its way in water through weathering of rocks. In saline and brackish waters its concentration is remarkably high and limits the biological diversity due to osmotic stress. Its salts are highly soluble in water and impart softness (in contrast to hardness).

High sodium content, in the form of chloride and sulphate, makes the water salty in taste and unfit for human consumption. High sodium content in irrigation water causes puddling of soil. As a result water intake of soil is reduced and it becomes hard in which germination of seed becomes difficult.

Material

i. Flamephotometer; ii. Whatman filter Paper; and iii. reagents, as given below:

Standard sodium solutions: Dissolve 2.5419 g of dried sodium chloride (AR) in distilled water to make 1 l of solution. This stock solution contains 1 g Na/l. Prepare various standard sodium solutions of different strengths (preferably in the ranges of 0 to 1, 0 to 10, and 0 to 100 mg Na/l) by diluting this stock solution with distilled water.

Method
Read the operation manual of flamephotometer at hand carefully. Set the filter for readings at 589 nm. Srart the compressor and light the burner of flamephotometer. Keep the air pressure at 5 lbs and adjust the gas feeder so as to have a blue sharp flame. Feed the standard sodium solution of the highest value in the range and adjust the flamephotometer to read full value of emission on the scale. Adjust the zero value of the meter by feeding distilled water. Now feed different standard sodium solutions within the range (i.e. 0-1, 0-10, or 0-100 mg Na/l) one by one and record the emission value for each. Plot a standard curve between concentration and emission of standard sodium solutions.

Filter the sample through filter paper and feed it in flamephotometer. Note the reading for sample and deduce sodium content of the sample in mg/l by comparing the value with standard curve.

3.2.18. Potassium

This cation occurs in natural waters in far lesser concentration than calcium, magnesium, and sodium. It behaves in the water as does sodium. Though found in small amounts it plays a vital role in the metabolism of freshwater environments and considered to be an important macronutrient.

Material
i. Flamephotometer; ii.Whatman filter paper; and iii. reagents, as given below:
Standard potassium solutions: Dissolve 1.9064 g of potassium chloride (AR) in distilled water to make the volume 1 l. This stock solution contains 1 g K/l. Prepare various standard potassium solutions of different strengths by diluting this stock solution with distilled water.

Method
Set the filter of flamephotometer for reading at 769 nm and proceed for determination of potassium in sample following the method described for the determination of sodium (see 3.2.17). Use standard potassium solutions for preparation of standard curve. Express the result of potassium content in mg/l.

3.2.19. Iron (Total, Ferrous, and Ferric)

Iron is found in all natural waters both in oxidized (ferric) and reduced (ferrous) forms. In anoxic reducing environments, like ground waters, most of the iron occurs in ferrous state (as ferrous bicarbonate under alkaline condition). Under aerobic conditions the ferrous state (ferrous bicarbonate) is quickly oxidized to ferric state (ferric hydroxide, a brown insoluble substance). During oxidation of ferrous bicarbonate to ferric hydroxide carbon dioxide is released.

Ferric iron is an important plant nutrient and considered to be quantitatively the most important trace metal for autotrophs because of its indispensability for many enzymes and redox processes. Some microorganisms (like **Creaothrix** and **Leptothrix**) are capable of utilizing dissolved iron (ferrous state) as an energy source and convert ferrous into ferric hydroxide.

1. Total iron

Material
i. Spectrophotometer; ii. laboratory glassware; and iii. reagents, as given below:
A. Hydrochloric acid: Concentrated, 12N.
B. Hydroxylamine hydrochloride solution: Dissolve 10 g of hydroxylamine hydrochloride in distilled water to make 100 ml of solution.

C. Ammonium acetate buffer solution: Dissolve 100 g of ammonium acetate in 60 ml of distilled water and add 280 ml glacial acetic acid.
D. Phenonthroline solution: Dissolve 50 mg of 1,10-phenonthroline monohydrate in 50 ml of distilled water by shaking and heating upto 80 °C in a water bath.
E. Standard iron solutions: Dilute 20 ml of sulphuric acid (concentrated) with 50 ml of distilled water, and dissolve in it 1.404 g of ferrous ammonium sulphate. Add 0.1N potassium permanganate solution (prepared by dissolving 3.16 g of $KMnO_4$ in distilled water to make 1 l of solution) slowly until a stable faint pink colour appears. Dilute to 1 l with distilled water. This stock solution contains 200 mg iron/l. Dilute the stock solution further to prepare a series of standard iron solutions, preferably in the range of 0 to 5 mg iron/l.

Method

Take 50 ml of sample in a conical flask and add 2 ml of hydrochloric acid (reagent A) and 1 ml of hydroxylamine hydrochloride solution (reagent B). Boil till the contents are reduced to about half the original volume. Cool and add 10 ml of ammonium acetate buffer solution (reagent C) and 2 ml of phenonthroline solution (reagent D). Add distilled water to make the volume 100 ml. Let stand for 10 minutes and then record the absorbance (S) on spectrophotometer at 510 nm using distilled water as blank.

Process the standard iron solutions of different concentrations (reagent E) in similar way and record the absorbance for each. Plot a standard curve from these values and deduce the total iron content of the sample in mg/l by comparing the absorbance of sample (S) with standard curve.

2. Ferrous iron (Soluble iron)

Material

i. Spectrophotometer; ii. laboratory glassware; and iii. reagents, as given below:

A. Hydrochloric acid: Concentrated, 12N.

B. Ammonium acetate buffer solution: Same as that used in total iron determination (see 3.2.19-1).

C. Phenonthroline solution: Same as that used in total iron determination (see 3.2.19-1).

Method

Take 50 ml of sample in a flask and acidify it with 1 ml of hydrochloric acid (reagent A). Add 10 ml of ammonium acetate buffer solution (reagent B) and 2 ml of phenonthroline solution (reagent C). Add distilled water to make the volume 100 ml. Wait for 10 minutes and then record the absorbance (S) on spectrophotometer at 510 nm using distilled water as blank.

Process the standard iron solutions of different concentrations (reagent E) in similar way and note the absorbance for each. Plot a standard curve using these values. Deduce the ferrous iron content of the sample in mg/l directly by comparing the absorbance of sample (S) with standard curve.

3. Ferric iron

Estimate the total iron and ferrous iron as discussed earlier (see 3.2.19-1&2). Compute the ferric iron in mg/l from these values as given below:

Ferric iron = Total iron - Ferrous iron

3.2.20. Nitrogen (Ammonia, Nitrite, Nitrate, and Organic nitrogen)

Nitrogen, which forms a major constituent (80%) of atmosphere, is found in small amounts in water because of low solubility of molecular nitrogen in water. Only a few prokaryotes can utilize this molecular nitrogen in biosynthesis. Besides this, nitrogen also occurs in small amounts in water as bound forms, like ammonia, nitrite, nitrate, and organic nitrogen (viz.,urea, amino acids, nucleic acids etc.). The source of such bound forms of nitrogen are both autochthonous and allochthonous.

1. Ammonia

Ammonia in the water is released as an end product of decomposition of organic matter and also as an excretory product of some aquatic animals. It dissolves in water to form ammonium hydroxide which further dissociates into ammonium (NH_4^+) and hydroxyl (OH^-) ions.

$$NH_3 + H_2O \longrightarrow NH_4OH \longrightarrow NH_4^+ + OH^-$$

Domestic wastes being often rich in nitrogenous organic matter, and many industrial effluents add to the ammonia load in water leading to toxic levels at certain times. Aquatic autotrophs are capable of utilizing ammonium ions at a fast rate.

I. Colorimetric method

Material

i. Spectrophotometer; ii. laboratory glassware; and iii. reagents, as given below:

A. Phenol-nitroprusside solution: Dissolve 15 g of phenol in 500 ml of distilled water. Add 1 ml of freshly prepared 1.5% w/v aqueous solution of sodium nitroprusside.

B. Alkaline hypochlorite solution: Dissolve 10 g of sodium hydroxide in some distilled water and add 2.7 ml of 10% solution of hypochlorite. Make

the volume 500 ml by adding distilled water.
C. Standard ammonium chloride solutions: Dissolve 3.82 g of anhydrous ammonium chloride in distilled water to make 1 l of solution. This stock solution contains 1 g NH_4^+-N/l (or 1.22 g NH_3 /l). Prepare a range of standard solutions of different concentrations diluting this stock solution with distilled water.

Note that all reagents should be prepared in ammonia-free distilled water. To remove the traces of ammonia distil the water after adding a small quantity of sulphuric acid to it.

Method

Take 20 ml of sample in a volumetric flask of 25 ml. Add 2 ml of phenol-nitroprusside solution (reagent A) and 2 ml of alkaline hypochlorite solution (reagent B). Make up the volume of contents to 25 ml by adding ammonia-free distilled water. Keep it in a dark place at 25 °C temperature for about 1 hour. Record the absorbance (S) on spectrophotometer at 635 nm. Use distilled water as blank.

Process the standard ammonium chloride solutions of different concentrations (reagent C) in similar way and record the absorbance for each. Plot a standard curve from these values and deduce the concentration of ammonium ions in sample in mg NH_4-N/l by comparing the absorbance of sample (S) with standard curve.

II. Volumetric method

Material

i. Micro-Kjeldahl distillation assembly; ii. laboratory glassware; and iii. reagents , as given below:

A. Hydrochloric acid (0.01N): Dilute 8.34 ml of 12N concentrated hydrochloric acid with distilled water to prepare 100 ml of 1.0N hydrochloric acid.

Dilute 100 ml of this 1.0N HCl with distilled water to prepare 1 l of 0.1N HCl. Take 100 ml of 0.1N HCl and dilute it to 1 l with distilled water to get 0.01N HCl.

B. Boric acid cum indicator solution: (a). Dissolve 4 g of boric acid in 100 ml of warm distilled water. (b). Prepare 0.5% bromocresol green solution in ethyl alcohol. (c). Prepare 0.1% methyl red solution in ethyl alcohol. (d). Mix bromocresol green (b) and methyl red (c) solutions in the ratio of 2:1 to make a mixed indicator. (e). Add 5 ml of this mixed indicator (d) to 100 ml of boric acid solution (a). If the colour of solution becomes blue add 0.01N hydrochloric acid (reagent A) until it turns faint pink to brown.

C. Borax buffer solution: Add 4 g of borax crystals to 100 ml of distilled water and heat to dissolve.

Method

Take 50 ml of sample in micro-Kjeldahl distillation assembly (Fig.13) through way **A** and add 1 ml of borax buffer solution (reagent C). Put 5 ml of boric acid cum indicator solution (reagent B) in a conical flask **F**. Place it below the condensor **C** so that the tip of outlet of condensor is dipped in contents of conical flask. Heat the boiling flask **E** containing distilled water so that the steam passes into sample through chamber **D**. Continue the distillation until about 40 ml of distillate is collected in the conical flask. Remove the conical flask having distillate which turns blue due to dissolution of ammonia. Cool the boiling flask **E** so that all wastes are sucked into chamber **D** and removed through tap **G**. Titrate the distillate in conical flask against 0.01N hydrochloric acid (reagent A). Turning of blue colour to faint pink or brown indicates the end point. Run a distilled water blank in similar way.

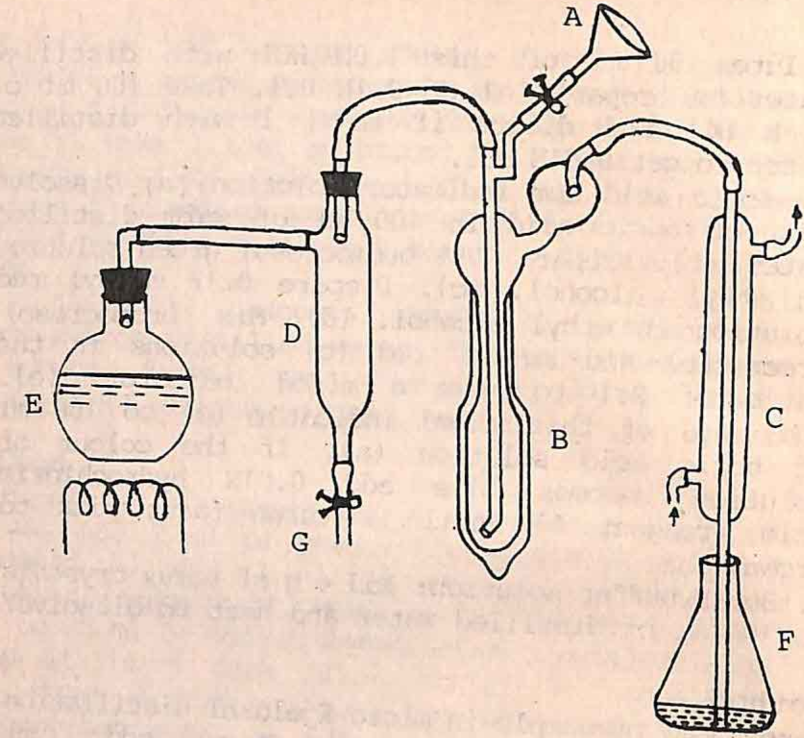

Fig.13. Micro-Kjeldahl assembly.

Calculation

$$NH_4\text{-}N\ (mg/l) = \frac{A - B \times N \times 1000 \times 14}{V}$$

Where, A = volume of titrant used against sample (ml); B = volume of titrant used against blank (ml); N = normality of titrant (0.01); and V = volume of sample (ml).

2. Nitrite

Nitrite is the partially oxidized form of nitrogen found in very low concentration in natural waters. It has no mineral source in water, but occurs as an intermediate form during denitrification and nitrification reactions.

NITRIFICATION

$$\text{Ammonia} \rightleftharpoons \text{Nitrite} \rightleftharpoons \text{Nitrate}$$

DENITRIFICATION

As such, nitrite in water is formed either by oxidation of ammonia (by aerobic nitrifying bacteria, e.g. **Nitrosomonas**) or by reduction of nitrates (by facultative anaerobic denitrifying bacteria, e.g. **Pseudomonas**). Presence of even minute quantity of nitrite in water is indicative of organic pollution and prevailing low oxygen concentration. At high concentration it may cause blue-baby disease.

I. EDTA method

Material

i. Spectrophotometer; ii. laboratory glassware; and iii.reagents, as given below:

A. EDTA solution: Dissolve 500 mg of disodium salt of EDTA (ethylene diamine tetra-acetic acid) in distilled water to prepare 100 ml of solution.

B. Sulphanilic acid solution: Dissolve 600 mg of sulphanilic acid in about 70 ml of warm distilled water. Cool, add 20 ml of hydrochloric acid (concentrated), and make the volume of contents to 100 ml by further adding distilled water.

C. a-Naphthylamine hydrochloride solution: Dissolve 600 mg of a-naphthylamine hydrochloride in a little distiled water. Add 1 ml of hydrochloric acid (concentrated) and make the volume to 100 ml by further adding distilled water. If on storage a precipitate appears, filter the reagent before use.

D. Sodium acetate solution: Dissolve 27.2 g of sodium acetate in distilled water to make 100 ml of solution.

E. Standard nitrite solutions: Dissolve 1.232 g of sodium nitrite in distilled water to prepare 1 l of solution. Take 4 ml of this solution and add distilled water to prepare 1 l of stock solution having 1 mg NO_2-N/l (or 2.290 mg NO_2 ions/l). Prepare standard nitrite solutions of various concentrations (preferably in the range from 0.0 to 1.0 mg NO_2-N/l at the intervals of 0.1 mg NO_2-N/l) by diluting stock solution with distilled water.

Method

Take 50 ml of filtered sample in an Erlenmeyer flask and add 1 ml of each EDTA solution (reagent A), sulphanilic acid (reagent B) and a-naphthylamine hydrochloride solution (reagent C) one after the other. An appearance of wine red colour indicates the presence of nitrites. Record the absorbance (S) of this solution on spectrophotometer at 520 nm. Use distilled water as blank.

Run standard nitrite solutions (reagent E) in similar way and record the absorbance for different concentrations. Plot a standard curve between absorbances and concentrations of standard solutions and deduce the nitrite-nitrogen content of sample by comparing its absorbance (S) with the standard curve. Express the result as mg NO_2-N/l.

II. Sulphanilamide method

Material

i. Spectrophotometer; ii. laboratory glassware; and iii. reagents, as given below:

A. Sulphanilamide solution: Dissolve 1 g of sulphanilamide in 100 ml of 10% hydrochloric acid.

B. N-1 naphthyl ehtylene diamine hydrochloride solution: Dissolve 0.1 g of N-1 naphthyl ethylene diamine hydrochloride in 100 ml of distilled water.

C. Standard nitrite solutions: Same as those used in EDTA method of nitrite determination (see 3.2.20-2-I).

Method

Take 45 ml of sample in a volumetric flask of 50 ml. Add 1 ml of sulphanilamide solution (reagent A) and after 3 minutes 1 ml of N-1 naphthyl ethylene diamine hydrochloride solution (reagent B). Make up the volume to 50 ml by further adding distilled water. Shake thoroughly and measure the absorbance (S) on spectrophotometer at 543 nm. Use distilled water as blank.

Process the standard nitrite solutions (reagent C) in similar way and record their absorbances. Plot a standard curve between absorbances and concentrations of standard solutions. Deduce the nitrite-nitrogen of sample by comparing its absorbance (S) with standard curve, and express the result in mg NO_2-N/l.

3. Nitrate

Nitrate is the highest oxidized form of nitrogen and in water its most important source is biological oxidation of nitrogenous organic matter of both outochthonous and allochthonous origin. Domestic sewage and agricultural runoff are the chief sources of allochthonous nitrogenous organic matter. Metabolic wastes of aquatic community and dead organisms add to the autochthonous nitrogenous organic matter. Nitrifying bacteria (aminifying bacteria, **Nitrosomonas, Nitrobactor**) play significant role in oxidation of such organic matter. Certain nitrogen-fixing bacteria (viz., **Azobactor**) and algae (viz., blue-greens like **Anabaena, Nostoc**) have capacity to fix molecular nitrogen in nitrates. In ground water nitrates may find way through leaching from soil and at times by contamination.

The high concentration of nitrate in water is indicative of pollution. This is an important plant nutrient, when present in excess it causes ubiquitous growth of algae, often present in blooms. High nitrate content (> 40 mg NO_3-N/l) may cause blue-baby disease.

I. Phenol disulphonic acid method

Material

i. Spectrophotometer; ii. laboratory glassware; iii. hot water bath; and iv. reagents, as given below:

A. Phenol disulphonic acid: Dissolve 25 g of white phenol in 150 ml of sulphuric acid (concentrated) and further add 85 ml of sulphuric acid (concentrated). Heat for about 2 hours on a water bath, cool, and keep the solution in a dark bottle.

B. Potassium hydroxide solution (12N): Dissolve 336.5 g of potassium hydroxide in distilled water to make the volume 500 ml.

C.Standard nitrate solutions: Dissolve 0.722 g of anhydrous potassium nitrate in distilled water to prepare 1 l of stock solution. This stock solution contains 100 mg NO_3-N/l(or 443 mg NO_3ions/l). Prepare standard nitrate solutions of various strengths (preferably in the range from 0.0 to 1.0 mg NO_3-N/l at the intervals of 0.1 mg NO_3-N/l) by diluting stock solution with distilled water.

Method

Take 25 ml of sample in a porcelain basin and evaporate it to dryness on a hot water bath. Add 0.5 ml of phenol disulphonic acid (reagent A) to the residue and dissolve the latter with the help of a glass spatula. Add 5 ml of distilled water and 1.5 ml of potassium hydroxide solution (reagent B). Stirr for thorough mixing. Take the supernatant of yellow colour and read its absorbance (S) on psectrophotometer at 410 nm. Use distilled water as blank.

Process the standard nitrate solutions (reagent C) in similar manner and note the absorbance for each. Plot a standard curve between absorbances and concentrations of various standard solutions. Deduce the value of nitrate and nitrogen in the sample by comparing the absorbance of sample (S) with the standard curve and express the result in mg NO_3-N/l.

II. Brucine method

Material

i. Spectrophotometer; ii. laboratory glassware; iii. hot water bath; and iv. reagents, as given below:

A. Brucine-sulphanilic acid solution: Dissolve 1 g of brucine-sulphate and 0.1 g of sulphanilic acid in about 50 ml of warm distilled water. Add 3 ml of concentrated hydrochloric acid and dilute the solution to 100 ml by further adding distilled water.

B. Sulphuric acid (diluted): In 125 ml of distilled water add slowly the 500 ml of concentrated sulphuric acid and cool.

C. Sodium chloride solution: Dissolve 300 g of sodium chloride in distilled water to prepare 1 l of solution.

D. Standard nitrate solutions: Same as those used in phenol disulphonic acid method of nitrate estimation (see 3.2.20-3-I).

Method

Take 10 ml of sample in an Erlenmeyer flask and add 2 ml of sodium chloride solution (reagent C). Shake the contents and place the flask in a cool water bath. Add slowly 10 ml of sulphuric acid (reagent B) and 0.5 ml of brucine-sulphanilic acid solution (reagent A). Shake well and put the flask in a hot water bath with boiling water for 20 minutes. Cool the contents and record the absorbance (S) on spectrophotometer at 410 nm. Use distilled water as blank.

Run the standard nitrate solutions (reagent D) in similar manner and note the absorbance for each. Plot a standard curve between absorbances and concentrations of various standard solutions. Deduce the value of nitrate-nitrogen in sample by comparing the absorbance of sample (S) with the standard curve and express the result in mg NO_3-N/l.

4. Organic nitrogen (Total, Dissolved, and Particulate)

In water, organic nitrogen is found in two forms, particulate and dissolved. Particulate organic nitrogen (PON) occurs as bound nitrogen in the protoplasm of aquatic organisms, while dissolved organic nitrogen (DON) as products like amino acids and urea etc. In organically rich (eutrophic) waters DON often exceeds PON.

a. Total organic nitrogen (TON)

Material

i. Micro-Kheldahl distillation assembly; ii. laboratory glassware; and iii. reagents, as given below:

A. Digestion mixture: Dissolve 16.25 g of potassium sulphate in 200 ml of distilled water. Add 0.4 g of murcuric oxide and slowly 25 ml of sulphuric acid (concentrated). Further add distilled water to make the volume 250 ml.

B.Hypo solution: Dissolve 50 g of sodium hydroxide in 200 ml of distilled water and add 10 g of sodium thiosulphate. Make up the volume to 250 ml by adding distilled water.

C. Boric acid solution: Dissolve 1 g of boric acid in distilled water to make 100 ml of solution.

D. Mixed indicator: (a). Prepare 0.1% methyl red solution in 95% ethyl alcohol. (b). Prepare 0.5% bromocresol green solution in 95% ethyl alcohol. Mix the methyl red (a) and bromocresol green (b) solutions in 1:2 ratio.

E. Hydrochloric acid (0.01N): Dilute 8.34 ml of hydrochloric acid (concentrated, 12N) with distilled water to prepare 100 ml of 1.0N hydrochloric acid. Dilute 100 ml of this 1.0N HCl with distilled water to prepare 1 l of 0.1N HCl. Take 100 ml of 0.1N HCl and dilute to 1 l with distilled water to get 0.01N HCl.

Method

Take 200 ml of sample in an evaporating dish and evaporate to dryness. Add 4 ml of digestion mixture (reagent A) to the residue and dissolve it in about 20 ml of distilled water. Heat the solution to fuming for over 15 minutes and cool. Transfer the digest to micro-Kjeldahl distillation assembly (Fig.13) through way **A** and add about 3.5 ml of hypo solution (reagent B). Take 5 ml of boric acid solution (reagent C) containing 2-3 drops of mixed indicator (reagent D) in a conical flask **F** placed below the condensor **C** so that the tip of outlet of condensor is dipped in contents of conical flask. Heat the boiling flask **E** so that the steam passes into sample through chamber **D.** Continue the distillation for about 10 minutes. Remove the conical flask **F** having distillate. Cool the boiling flask **E** so that all wastes are sucked into chamber **D** and removed through tap **G** . Titrate the distillate against hydrochloric acid (reagent E). Turning of blue colour to pink indicates end point. Run a distilled water blank in similar way.

Calculation

$$\text{TON (mg/l)} = \frac{A - B \times N \times 1000 \times 14}{V}$$

Where, A = volume of titrant used against sample (ml); B = volume of titrant used against blank (ml); N = normality of titrant (0.01); and V = volume of sample (ml).

b. Dissolved organic nitrogen (DON)

Filter the sample through a millipore filter paper using a filteration unit and process the filterate in a manner similar to that employed for estimation of total organic nitrogen (TON; see 3.2.20-4-a). Express the result of dissolved organic nitrogen in mg/l.

c. Particulate organic nitrogen (PON)

Determine the total and dissolved organic nitrogen as discussed earlier (see 3.2.20-4-a & b) and compute the particulate organic nitrogen as follows:

$$\text{PON (mg/l)} = \text{TON} - \text{DON}$$

Where, TON = total organic nitrogen (mg/l); and DON = dissolved organic nitrogen (mg/l).

3.2.21. Phosphorus (Total, Inorganic and Organic)

Phosphorus bound in rocks is generally insoluble in water, so in natural waters its content is low. Domestic and industrial effluents,and agricultural runoff are major sources of phosphorus in water, hence its high concentration is indicative of pollution. In water, phosphorus occurs both in inorganic and organic forms but since over 85% of total phosphorus is usually present in organic form (as bound in the organismal matter) only inorganic phosphorus (as orthophosphate) plays a dynamic role in an aquatic ecosystem. Under oxidizing conditions (high redox potential) it is precipitated and lost to the sediments resulting in depletion of phosphorus in water. On the other hand, under reducing conditions (low redox potential) some phosphorus returns to water in soluble form.

Though present in low concentration, phosphorus is one of the most important nutrients limiting growth of autotrophs and so biological productivity of the system. High phosphorus content causes increased algal growth, often as blooms, till nitrogen becomes limiting. Blue-greens, however, continue to grow as dominant algae because of their ability to utilize molecular nitrogen. Besides sedimentation, high uptake by phytoplankton is one of the reasons of fast depletion of phosphorus from water.

1. Total phosphorus

Material

i. Spectrophotometer; ii. laboratory glassware; iii. hot plate; and iv. reagents, as given below:

A. Perchloric acid (70%).

B. Phenolphthalein indicator: Dissolve 1.0 g of phenolphthalein in 100 ml of ethyl alcohol and add 100 ml of distilled water.

C. Sodium hydroxide solution (1N): Dissolve 4.0 g of sodium hydroxide in distilled water to prepare 100 ml of solution.

D. Ammonium molybdate solution: Add 62 ml of sulphuric acid (concentrated) slowly to 80 ml of distilled water and let cool. Dissolve separately 5 g of ammonium molybdate in 35 ml of distilled water and mix it with sulphuric acid solution. Add distilled water to make the solution 200 ml.

E. Stannous chloride solution: Dissolve 0.5 g of stannous chloride in 2 ml of hydrochloric acid (concentrated) and dilute to 20 ml with distilled water. Use fresh.

F. Standard phosphate solutions: Dissolve 4.388 g of dried anhydrous potassium hydrogen phosphate in distilled water to make the volume 1 l. Take 10 ml of this solution and add distilled water to make 1 l of stock solution containing 1 mg P/l. Prepare standard phosphorus solutions of various strengths (preferably in the range of 0.0 to 1.0 mg P/l at intervals of 0.1 mg P/l) by diluting the stock solution with distilled water.

Method

Take 25 ml of sample in an Erlenmeyer flask and evaporate to dryness. Cool and dissolve the residue in 1 ml of perchloric acid (reagent A). Heat the flask gently so that the contents become colourless. Cool and add 10 ml of distilled water and 2 drops of phenolphthalein indicator (reagent B). Titrate against sodium hydroxide solution (reagent C) until the appearance of slight pink colour. Make up the volume to 25 ml by adding distilled water. Add 1 ml of ammonium molybdate solution (reagent D) and 3 drops of stannous chloride solution (reagent E). A blue colour will appear. Wait for 10 minutes (never more than 15 minutes) and record absorbance (S) on spectrophotometer at 690 nm. Run simultaneously a distilled water blank in similar manner.

Process the standard phosphorus solutions of different strengths (reagent E) in similar manner and plot a standard curve between absorbances and concentrations of standard phosphorus solutions. Deduce the total phosphorus content of sample by comparing its absorbance (S) with standard curve and express the result of total phosphorus in mg/l.

The total particulate phosphorus can be estimated as a difference between the concentration of total phosphorus in unfiltered and filtered samples.

2. Inorganic phosphorus (Orthophosphate or Reactive soluble phosphorus)

Material

Same as that used for estimation of total phosphorus (see 3.2.21-1), excepting hot plate and the following reagents:
A. Perchloric acid; B. Phenolphthalein indicator; and C. Sodium hydroxide solution.

Method

Take 25 ml of sample in a flask and add to it 1 ml of ammonium molybdate solution (reagent D) and 3 drops of stannous chloride solution (reagent E). A blue colour will appear. Wait for 10 minutes (never more than 15 minutes) and record absorbance (S) on spectrophotometer at 690 nm. Run a distilled water blank in similar manner.

Process the standard phosphorus solutions of different strengths (reagent F) in similar manner and plot a standard curve between absorbances and concentrations of standard phosphorus solutions. Deduce the inorganic phosphorus (as orthophosphate) content of sample by comparing its absorbance (S) with standard curve and express the result in mg PO_4-P/l.

3. Organic phosphorus

Organic phosphorus content of water can be determined through following calculation employing values of total and inorganic phosphorus.

$$\text{Organic phosphorus (mg/l)} = TP - IP$$

Where, TP = total phosphorus (mg/l); and IP = inorganic phosphorus (mg/l).

3.2.22. Silica

Silica in water is found as silicate which is a soluble reactive form. Its concentration in natural waters is considerably high, ranging from 1 to 30 mg SiO_3-Si/l or still more. At high temperature and pH the solubility of silica is greatly increased.

Since silica is an important structural constituent of diatoms and many sponges, it regulates their growth. The assimilation of silica and subsequent sedimentation by diatoms form the greatest sink of silica in water.

Material

i. Spectrophotometer; ii. laboratory glassware; and iii. reagents, as given below:

A. Hydrochloric acid (50%): Add 50 ml of hydrochloric acid (concentrated) to 50 ml of distilled water.

B. Ammonium molybdate solution: Dissolve 10 g of ammonium molybdate in distilled water to prepare 100 ml of solution. Adjust the pH between 7 and 8 by adding ammonium hydroxide. Keep the solution in a polyethylene bottle.

C. Oxalic acid solution: Dissolve 10 g of oxalic acid in distilled water to prepare 100 ml of solution.

D. Standard silica solutions: Add 671.4 mg of sodium fluorosilicate in a little distilled water and heat to dissolve. Further add distilled water to make the volume 1 l. This stock solution contains 100 mg SiO_3-Si/l. Dilute this stock solution with distilled water to prepare a series of standard silica solutions, preferably in the range of 0 to 50 mg SiO_3-Si/l at intervals of 5 mg SiO_3-Si/l.

Method

Take 50 ml of sample in an Erlenmeyer flask and add 1 ml of **hydrochloric acid** (reagent A) and 2 ml of **ammonium molybdate** solution (reagent B). Wait for about 10 minutes and add 1.5 ml of oxalic acid solution (reagent C). Mix thoroughly and record the absorbance (S) on spectrophotometer at 410 nm. Use distilled water as blank.

Run standard silica solutions (reagent D) in similar manner and record their absorbances. Plot a standard curve between absorbances and concentrations of standard solutions. Deduce the silica content of sample by comparing its absorbance (S) with standard curve. Express the result in mg SiO_3-Si/l.

3.2.23. Carbohydrate (Total, Dissolved, and Particulate)

Carbohydrates are synthesized by aquatic autotrophs and these may occur as intracellular matter as well as extracellular products. Besides pollution because of domestic wastes and effluents from industries, such as textile, distillery, and sugar mills etc., also add to the carbohydrate balance of a natural water. Carbohydrates play a vital role in the metabolism of aquatic community and their decomposition influences many physical and chemical factors within the system.

1. Total carbohydrate

Material

i. Spectrophotometer; ii. laboratory glassware; iii. hot water bath; and iv. reagents, as given below:

A. Anthrone reagent: Add 250 ml of sulphuric acid (concentrated) slowly to 100 ml of distilled water and dissolve 50 mg of anthrone in it. Store the reagent in dark bottle in the refrigerator.

B. Standard carbohydrate solutions: Dissolve 1 g of D-glucose in saturated benzoic acid. Prepare several dilutions of this stock solution to have a series of standard carbohydrate solutions.

Method

Take 1 ml of sample in a test tube and add 5 ml of anthrone reagent (reagent A). Heat the test tube in a hot water bath at boiling temperature for about 10 minutes. Cool the tube and record the absorbance (S) of contents on spectrophotometer at 620 nm. Run distilled water as blank.

Run standard carbohydrate solutions (reagent B) in similar manner and record the absorbance for each. Plot a standard curve between absorbances and concentrations of standard carbohydrate solutions. Deduce the carbohydrate content of sample by comparing its absorbance (S) with standard curve. Express the result of total carbohydrate content in mg/l.

2. Dissolved carbohydrate

In order to evaluate the dissolved carbohydrate content filter the sample through filter paper (Whatman No. 44) and determine the carbohydrate content in filterate following the method described for total carbohydrate (see 3.2.23-1). Express the result of dissolved carbohydrate content in mg/l.

3. Particulate carbohydrate

Determine the total and dissolved carbohydrates in mg/l as described earlier (see 3.2.23-1&2) and calculate the particulate carbohydrate as a difference of content of two carbohydrates.

3.2.24. Oil and grease

Oil and grease in natural waters are not of autochthonous origin but enter as a result of pollution. Being immisible in water and low in density they float and form a film on the surface, thereby precluding gaseous transactions through water surface and penetration of light to more or less extent. Such a film is specially dissastrous for the neustons (surface dwellers) and it impairs their normal respiration and movement. Many nektonic (free swimming) animals too come to surface to breath and they are adversely affected by oils and grease. Such substances also cause clogging of stomatal openings of macrophytes.

Material

i. Separatory funnel (500 ml); ii. filter paper; iii. hot water bath; and iv. reagents, as given below:

A. Sulphuric acid: Add 50 ml of sulphuric acid (concentrated) slowly to 100 ml of distilled water.

B. Petroleum ether (B.P.around 50 °C).

C. Ethyl alcohol (absolute).

Method

Collect 250 ml of sample in a wide mouth bottle and add 10 ml of sulphuric acid (reagent A), about 50 ml of petroleum ether (reagent B), and a little amount of ethyl alcohol (reagent C). Shake well and transfer the whole suspension to a separatory funnel. Allow to stand for some time so that the two layers, upper one of petroleum ether and lower one of water, become distinct. Discard lower layer of water from the separatory funnel. Drain out remaining petroleum ether from the separatory funnel, through a filter paper soaked in petroleum ether (reagent B), in a preweighed (Wi) glass beaker. Pass some more petroleum ether through filter paper so that no oil and grease remain stuck to the paper. Keep the beaker with contents in a hot water bath (never on flame) so as to evaporate the petroleum ether. Record the weight of beaker with residue in it (Wf).

Calculation

$$\text{Oil and grease (mg/l)} = \frac{(Wf - Wi) \times 1000}{V}$$

Where, Wi = initial weight of beaker (g); Wf = final weight of beaker with residue (g); and V= volume of sample (l).

3.2.25. Biochemical oxygen demand (BOD)

The rate of removal (i.e. consumption) of oxygen by microorganisms in aerobic degradation of the dissolved or even particulate organic matter in water is called biochemical oxygen demand (BOD) and it is used as an index of organic pollution in water. More the oxidizable organic matter present in water, more the amount of oxygen required to degrade it biologically, hence more the BOD.

BOD is evaluated by measuring oxygen concentration in sample before and after incubation in the dark at 20 °C for 5 days. Preliminary dilution and aeration of sample are usually necessary to ensure that not all the oxygen is consumed during incubation.

Material

i. BOD incubator; ii. BOD bottles; iii. other laboratory glassware; ans iv. reagents, as given below:

A. All reagents used in determination of dissolved oxygen as discussed earlier (see 3.2.5-I).

B. BOD-free water: Pass the deionised glass distilled water through a column of activated carbon and redistil it.

C. Phosphate buffer solution: Dissolve 33.4 g of $Na_2HPO_4.7H_2O$, 21.5 g of K_2HPO_4, 8.5 g of KH_2PO_4, and 1.7 g of NH_4Cl in BOD-free distilled water to prepare 1 l of solution. Adjust the pH to 7.2.

D. Magnesium sulphate solution: Dissolve 82.5 g of magnesium sulphate in BOD-free distilled water to prepare 1 l of solution.

E. Calcium chloride solution: Dissolve 27.5 g of anhydrous calcium chloride in BOD-free distilled water to prepare 1 l of solution.

F. Ferric chloride solution: Dissolve 0.25 g of ferric chloride in BOD-free distilled water.

G. Sulphuric acid (1N): Add 2.8 ml of sulphuric acid (concentrated) to 100 ml of BOD-free distilled water.

H. Sodium hydroxide solution (1N): Add 4 g ot sodium hydroxide in distilled water and make the volume 100 ml.

I. Allylthiourea solution: Dissolve 500 mg of allylthiourea in distilled water and make the volume 1 l.

Method

To prepare dilution water aerate the BOD-free distilled water in a glass container for about half an hour. Add per litre of this water 1 ml each of phosphate buffer solution (reagent C), magnesium sulphate solution (reagent D), calcium chloride solution (reagent E), and ferric chloride solution (reagent F).

Adjust the pH of sample to neutrality (7.0) using 1N sulphuric acid (reagent G) or 1N sodium hydroxide solution (reagent H) as the case may be. To ensure that not all the oxygen of sample is exhausted during incubation, dilute the sample with dilution water at the rate shown in Table 5, according to the expected BOD content of the sample. Fill two sets of BOD bottles with this water (sample) and add 1 ml of allylthiourea solution (reagent I) to each bottle. Determine the dissolved oxygen content (D_o) in one set immidiately following the Winkler's method of oxygen estimation as described earlier (see 3.2.5-I).

Incubate the other set of BOD bottles at 20 °C for five days in a BOD incubator. Take out the bottles after 5 days and determine immediately their dissolved oxygen content (D_5).

Table 5 . Dilution of sample required for various ranges of expected BOD.

Expected BOD (mg/l) in sample	Volume of sample (ml)/l of mixture	Dilution factor
0 - 6	1000	1
4 - 12	500	2
10 - 30	200	5
20 - 60	100	10
40 - 120	50	20
100 - 300	20	50
200 - 600	10	100
400 - 1200	5	200
1000 - 3000	2	500
2000 - 6000	1	1000
>6000	0.5	2000

Calculation

$$BOD_5\ (mg/l) = (D_o - D_5) \times \text{Dilution factor}$$

Where, D_o = initial dissolved oxygen in the sample (mg/l); and D_5 = dissoled oxygen left out in the sample after 5 days incubation (mg/l).

In tropical and subtropical regions where rate of metabolism is relatively high an incubation for 3 days at 27 °C (instead of 5 days at 20 °C) is in practice and BOD so determine is expressed as BOD_3 mg/l.

3.2.26. Chemical oxygen demand (COD)

Chemical oxygen demand (COD) is the measure of oxygen required in oxidizing the organic compounds present in water by means of chemical reactions involving oxidizing substances such as potassium dichromate and potassium permanganate. Potassium dichromate is the most suitable oxidant but for waters having more than 2 g/l of chlorides potassium permanganate is used, though the results are more variable because latter is self oxidizing.

The estimation of COD is of great importance for waters having unfavourable conditions for the growth of microorganisms, such as presence of toxic chemicals. In such waters BOD cannot be determined accurately. However, COD too is not a perfect index of organic compounds present in water because, in this reaction, many inorganic compounds are also oxidized, and at the same time some organic compounds remain unaffected.

Material

i. COD reflux unit (Fig.14) consisting of flat bottom flask with ground glass mouth (250 ml) and Liebig (straight tube, single surface) condensor (30 cm); ii. hot water bath or heating mantle; and iii. reagents, as given below:

A. Potassium dichromate solution (0.25N): Dissolve 12.259 g of potassium dichromate (dry,AR) in distilled water to prepare 1 l of solution.

B. Silver sulphate: Dry, powdered.

C. Murcuric sulphate: Dry, powdered.

D. Sulphuric acid: Concentrated.

E. Ferroin indicator solution: Dissolve 0.695 g of ferrous sulphate and 1.485 g of 1,10-phenonthroline in distilled

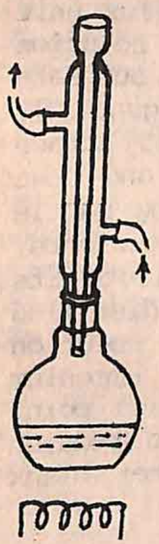

Fig. 14. COD reflux unit.

water to make 100 ml of indicator solution.

F. Ferrous ammonium sulphate solution (0.25N): Dissolve 98 g of ferrous ammonium sulphate in distilled water, add 20 ml of sulphuric acid (reagent D), cool and dilute to 1 l by further adding distilled water.

To standardized this solution, dilute 25 ml of potassium dichromate solution (reagent A) to about 250 ml with distilled water, add 20 ml of sulphuric acid (reagent D), and cool it. Add 5-6 drops of ferroin indicator solution (reagent E) and titrate against ferrous ammonium sulphate solution (reagent F). The colour changes from blue green to a reddish blue at end point.

$$\text{Normality of reagent F} = \frac{\text{VA} \times 0.25}{\text{VF}}$$

Where, VA = volume of reagent A (ml); and VF = volume of reagent F used (ml).

Method

Take 20 ml of sample in the flask of reflux unit and add 10 ml of potassium dichromate solution (reagent A), a pinch of each silver sulphate (reagent B) and murcuric sulphate (reagent C), and 30 ml of sulphuric acid (reagent D). Attach Liebig condensor to the mouth of flask and heat the flask on a hot water bath or heating mantle for at least 2 hours to reflux the contents. Cool the flask, detach from unit and dilute its contents to about 150 ml by adding distilled water. Add 2-3 drops of ferroin indicator solution (reagent E) and titrate against ferrous ammonium sulphate solution (reagent F). At the end point blue green colour of contents changes to reddish blue. Run simultaneously a distilled water blank in similar manner.

Calculation

$$\text{COD (mg/l)} = \frac{(B - A) \times N \times 1000 \times 8}{V}$$

Where, A = volume of titrant used against sample (ml); B = volume of titrant used against blank (ml); N = normality of titrant (0.25); and V = volume of sample (ml).

3.3. BIOLOGICAL PARAMETERS

3.3.1. Macrophytes

Macrophytes are the macroscopic plants in water and they grow mostly in shallow water region. A representative arrangement of macrophytes proceeding from shallow to deeper water is usually as follows:

(a) Zone of emergent vegetation: Rooted plants with main photosynthetic parts projecting above water surface. These get carbon dioxide from air but other raw materials from water and sediments. e.g. **Typha, Scirpus, Sagillaria** etc.

(b) Zone of vegetation with floating leaves: Rooted plants with their leaves floating on surface. The horizontal photosynthetic surface of these plants reduces largely the light penetration in water. e.g. Water lilies (**Nymphaea**).

(c) Zone of submergent vegetation: Rooted plants, completely or largely submerged. Leaves of such plants are thin and adapted for exchange of nutrients with the water. e.g. **Potomogeton, Ceratophyllum, Hydrilla, Vallisnaria, Chara** etc.

An other category of floating macrophytes is also very common in which crown of plant floats on the surface of water. Abundant in shallow region, these macrophytes are found floating on deeper water as well. e.g. **Eichornia.**

Macrophytes provide large surface which is harboured by a variety of microflora and fauna (periphyton).

They, especially those with floating leaves, cause shading effect in littoral zone creating adverse conditions for the growth of phytoplankton. Macrophytes are important primary producers in a body of water and they act as 'nutrient pump' since their roots often recover nutrients from deep in the anaerobic sediments. Many of the macrophytes, such as **Eichornia**, have been recognized as water weeds as they have nuisance value for the ecosystem. Some macrophytes are considered to be good indicators of water pollution. Technologies have been developed to utilize these 'weeds' as human, bird, and animal feed, biofertilizers, and source of energy.

1. Collection of macrophytes

Macrophytes can be collected using long handled hooks (Fig.15), nets (Fig.16), or by hand. For quantification of sample in a given area the floating or sinking type of quadrats of known sizes (viz., 1m x 1m or 0.5m x 0.5m) made up of PVC pipes or wood are used. These quadrats are placed in the macrophyte locality to mark the area from which sample is to be taken. Macrophytes taken out are thoroughly washed, excess water is absorbed on a cloth or filter paper, kept in polythene bags and brought to the laboratory in an ice box.

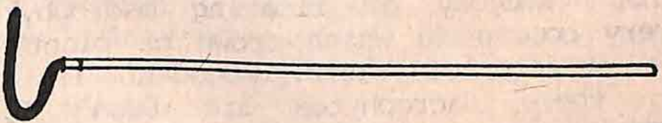

Fig.15. Long handled hook.

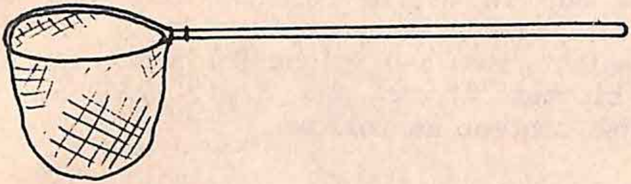

Fig.16. Long handled net.

2. Qualitative analysis of macrophytes

Material
i. Long handled nets and hooks; ii. plastic bucket; and iii. polythene bags etc.

Method
Collect the macrophytes as described earlier (see 3.1.1-1). Record their nature viz.,emergent, floating, submerged etc. Identify the macrophytes with the help of pertinent literature.

3. Quantitative analysis of macrophytes (Biomass, Moisture, Ash, and Organic matter)

Material
i. Quadrats, made up of PVC pipes or wood; ii. polythene bags; iii. ice box; physical balance and weights; v. oven, vi. crucible; and vii. furnace etc.

Method
Collect the macrophytes as described earlier (see 3.3.1-1). Sort out the plants species-wise. Weigh the plants of individual species and express as fresh weight of a species in g/quadrat. Put the plants in polythene bags and carry in an ice box to the laboratory. Dry the known weight of each species in an oven at 105 °C till constant weight is attained. Weigh the dried sample (plant

material) and calculate the dry weight of a species in g/quadrat. Take a known weight of sample (D) in a silica crucible and incinerate it at 550 °C for a day in muffle furnace. Cool and weigh the ash (A). From the average value of fresh weight, dry weight, and ash weight for a species calculate the biomass (fresh and dry), moisture content, and ash content as follows.

Calculation

$$\text{Fresh biomass } (g/m^2) = \frac{\text{Fresh weight (g/quadrat)}}{\text{Area of quadrat } (m^2)}$$

$$\text{Dry biomass } (g/m^2) = \frac{\text{Dry weight (g/quadrat)}}{\text{Area of quadrat } (m^2)}$$

$$\text{Moisture content } (\%) = \frac{\overset{\text{Fresh}}{\text{weight}} - \overset{\text{Dry}}{\text{weight}}}{\text{Fresh weight}} \times 100$$

$$\underset{(\%\text{ of dry weight})}{\text{Ash content}} = \frac{\text{Ash weight (A)}}{\text{Dry weight (D)}} \times 100$$

Ash-free dry weight= Dry weight(D) - Ash weight(A)
(or organic matter)

3.3.2. Phytoplankton

Phytoplankton are floating microscopic autotrophs whose movements are more or less dependent on currents. These consist of algae (mainly members of Chlorophyceae, Cyanophyceae, and Bacillariophyceae) and algae-like green flagellates. Even though most of the forms are microscopic, yet they often impart green colour to the water. Phytoplankton may exceed rooted plants in food production per unit area. Many of such forms have processes or other adaptations to aid in floating, while turbulence and upward-current movements of water aid in keeping phytoplankton near the surface where photosynthesis is most effective. According to the size, phytoplankton are classified as follows:

Ultraplankton	0.5 to 10 µm
Nannoplankton	10 to 50 µm
Microplankton (Net plankton)	50 to 500 µm
Macroplankton	> 500 µm

Phytoplankton, because of their role as primary producers in an aquatic ecosystem, are the subject of great interest. Their qualitative and quantitative estimates provide good indices of water quality and capacity of water to sustain heterotrophic communities. Phytoplankton also play a vital role in the biological treatment of organic wastes in water, as during mineralization of organic matter by bacteria the oxygen is supplied by them. Therefore, evaluation of polulation diversity, periodicity and succession, spatial and temporal patterns, biomass and productivity of phytoplankton is very important in monitoring of a body of water.

1. Collection and preservation of phytoplankton

Larger phytoplankton may be collected by filtering a known amount of water through a plankton net made up of bolting silk (No. 25; i.e. mesh size 55 µm). Because through this net only micro and macroplankton can be collected and many minute forms will pass out resulting in underestimation of population, this method of phytoplankton collection can nevertheless be quite useful .

Direct concentration of phytoplankton from water sample is considered to be a better method of collection. In this 1 l (or more or less, depending upon richness of plankton) of water sample is taken in a wide mouth glass bottle. To this Lugol's iodine solution* @ 10 ml/l of sample is added. Lugol's iodine solution speeds up the rate of sedimentation of phytoplankton, provides them stain, and preserves their flagella and cilia; however, this being not a good long term preservative sample should be preserved in 4% formaldehyde. Sample is allowed to stand for a day or more to ensure complete sedimentation of phytoplankton. The clear supernatant is removed with the help of a pipette creating little disturbance. Supernatant is examined under microscope to ensure that it is phytoplankton-free. Make up the sediment (phytoplankton) to a fixed volume (say 10,50, or 100 ml) and record this concentration factor. This concentrated phytopankton sample represents the population in 1 l (or original volume of sample) of water.

2. Qualitative analysis of phytoplankton

Collect the phytoplankton as described earlier (see 3.3.2 -1). Identify them under a microscope, preferably an inverted plankton microscope, using pertinent literature.

3. Measurement of phytoplankton population

Collect the phytoplankton as described earlier (see 3.3.2-1) and count them by one of the methods described below. Because many phytoplankters occur in colonies (viz., **Microcystis, Merismopedia, Aphanocapsa**), many are solitary cells (viz., **Nitzschia, Chlorella**), and many are multicelled filamentous (viz:, **Oscillatoria, Lingbya, Anabaena**), this makes it difficult to express the results of enumeration as cell/l. The results are thus more conveniently expressed as units/l where, a unit represents to a colony of any size for colonial phytoplankter, a definite length (say 10 µm) for a filamentous phytoplankter, or a cell for a solitary single-celled phytoplankter. The degree of abundance for individual phytoplankters may be recorded as follows:

---

* To prepare Lugol's iodine solution dissolve 10 g of neutral potassium iodide in 20 ml of distilled water and add 5 g of iodine crystals, 50 ml of additional distilled water and 5 g of sodium acetate.

Rare occurrence	$\lessdot 15 \times 10^3$ units/l
Frequent occurrence	16 to 99×10^3 units/l
Subdominant	100 to 999×10^3 units/l
Dominant	$\gtrdot 1000 \times 10^3$ units/l

I. Sedgwick-Rafter cell method

Material

i. Sedgwick-Rafter cell; ii. dropper; and iii. compound microscope, preferably inverted plankton microscope.

Method

Sedgewick-Rafter cell is a slide with a rectangular cavity (50 mm x 20 mm x 1 mm) of volume 1 cm^3 (i.e. 1 ml).

Shake well the concentrated sample and quickly transfer 1 ml of it in the cavity of Sedgwick-Rafter cell with the help of a dropper. Cover the cavity with a cover slip avoiding any air bubble. Allow the planktons to settle and count them under a microscope by moving the cell horizontally and vertically. Study a good number of replicates and calculate the average count per ml.

Calculation

$$\text{Phytoplankton (units/l)} = \frac{N \times C}{V}$$

Where, N = number of phytoplankton counted in 1 ml of concentrate; C = total volume of concentrate (ml); V = total volume of sample in l (this represents total volume of water filtered through net when collection is made by filtration; or total volume of water taken in bottle for sedimentation when collection is made by sedimentation method).

II. Lackey's drop method

Material

i. Glass slide; ii. cover slip; iii. graduated medicinal dropper; and iv. compound microscope, preferably inverted plankton microscope.

Method

This mathod is simple and especially useful when phytoplankton density is considerably high. Shake the concentrated sample of phytoplankton and quickly transfer 0.1 ml volume of it on a glass slide with the help of a graduated dropper. Cover the drop carefully with a cover slip of suitable size so that no fraction of sample runs out. Study and count the phytoplankton present under cover slip. Study a good number of replicates and calculate the average count per 0.1 ml.

Calculation

$$\text{Phytoplankton (units/l)} = \frac{n \times C}{V} \times 10$$

Where, n = number of phytoplankton counted in 0.1 ml of concentrate; C = total volume of concentrate (ml); and V = total volume of sample in l (this represents total volume of water filtered through net when collection is made by filteration; or total volume of water taken in bottle for sedimentation when collection is made by sedimentation method).

4. Measurement of phytoplankton biomass

Because the cells and colonies of phytoplankton differ greatly in their size, mere count of them is not enough for all the purposes. Therefore, the average cell volumes and the biomass of phytoplankton are considered to be better parameters for precise quantification.

Material

i. Material as required for plankton counting (see 3.3.2-3); and ii. ocular and stage micrometers.

Method

Make a count of various species of phytoplankton as described earlier (see 3.3.2-3). Make the measurements of diameter (width) and height (length) of some of the units (cells/colonies/filaments) of each species in micron using ocular and stage micrometers with a microscope. Also record the shape (viz., cylindrical, conical, spherical, or hemispherical) of the unit of each species.

Calculation

Volume of a unit of a species (UV; u^3) is calculated on the basis of its shape, employing one of the following formulae:

Volume of a cylindrical unit = $\pi r^2 h$
Volume of a conical unit = $(\pi r^2 h)/3$
Volume of a spherical unit = $(4 r^3 \pi)/3$
Volume of a hemispherical unit = $(4 r^3 \pi)/6$

Where, r = radius (μ); h = height or length (μ); and $\pi = 3.142$.

Total volume of all the units of a species (TUV) per litre of water is calculated as follows:

$$\text{TUV } (\mu^3/l) = \text{UV} \times n$$

Where, UV = volume of a unit of a species (μ^3); and n = number of units of same species per l of water.

Total plankton volume (TPV) per l of water is calculated as a sum of total unit volumes (TUV) of different species, as follows:

$$TPV\ (\mu^3/l) = (TUV)_1 + (TUV)_2 \ldots\ldots + (TUV)_n$$

Assuming the density of plankton and water to be equal (i.e. 1), the total planktonic biomass (TPB) is calculated as follows:

$$TPB\ (g/l) = \frac{TPV}{10^{12}}$$

The total biomass of a species is calculated as follows:

$$\text{Total biomass of a species } (g/l) = \frac{TUV}{10^{12}}$$

3.3.3. Zooplankton

Zooplankton are heterogeneous assemblage of minute floating animal forms found in water. They may bear some locomotory structures but are not capable of moving against the water currents, therefore, as in case of phytoplankton, their movement is largely at the mercy of water movements. They usually act as primary consumers and constitute an important link between primary producers (phytoplankton) and higher consumers (like carnivore fish) in aquatic food chains. Zooplankton are represented by a wide array of animal groups, of which members of Protozoa, Rotifera, Copepoda, Cladocera, and Ostracoda are often dominating. Reproductive bodies (like gemmules and eggs etc.) and larval stages of large variety of animals including fish, are also found in zooplanktonic collections. The size-wise classification, as given for phytoplankton (see 3.3.2), is equally valid for zooplankton too.

1. Collection and preservation of zooplankton

Zooplankton may be collected by filtering a known volume of water through zooplankton net made up of bolting silk (No. 25; mesh size 55 μ). The surface water for this purpose may be collected with the help of a plastic bucket or jug of known volume but for sub-surface waters Von Dorn or pump samplers (see 2.3) are considered to be better devices.

When overall zooplanktonic composition and population, irrespective of the depth, are to be analysed, a vertical towing of plankton net is very useful. For this, a plankton net weighted at the base is lowered in the water to a known depth with the help of a graduated cord and is then pulled slowely (say 10 cm/sec) out. Plankton collected in the plankton bottle fitted at the end of the net are taken out. The volume of water filtered through plankton net is calculated employing following formula:

$$V = \pi r^2 h$$

Where, V = volume of water (l); π = a constant (3.142); r = radius of the mouth of plankton net (m); and h = depth upto which the net was lowered (m).

When the net is towed horizontally in water (as hanging horizontally down to a powered boat) the **h** in this formula represents the distance travelled by net (m).

Transfer the zooplankton collected to a small bottle (50-100 ml) and preserve in 4% formaldehyde.

2. Qualitative analysis of zooplankton

Collct the zooplankton as described above (see 3.3.3-1) and identify them under a microscope, preferably inverted plankton microscope, using pertinent literature.

3. Measurement of zooplankton population

Collect the zooplankton as described earlier (see 3.3.3-1) and enumerate various species and total zooplankton following the Sedgwick-Rafter cell method (see 3.3.2-3-I). Express the results in organisms/l.

4. Measurement of zooplankton biomass

Collect and preserve the zooplankton as described earlier (see 3.3.3-1). Transfer them to a finely graduated measuring cylinder or tube of suitably small volume. Allow the sample to stand for complete sedimentation; if necessary, centrifuge it. Note the volume of zooplankton (Vz) in ml and assuming the density of plankton equal to that of water (i.e. 1), calculate the biomass as follows:

$$\text{Zooplankton biomass (g/l)} = \frac{Vz}{Vs}$$

Where, Vz = volume of zooplankton (ml); and Vs = volume of water filtered to procure the zooplankton (l).

3.3.4. Periphyton (Biotecton)

Periphyton or aufwuchs are the organisms (both plant and animal) attached or clinging to the submerged plant or other surfaces projecting above the bottom. Among plants algae contribute mainly to such a community while among animals diverse groups from Protozoa to vertebrate eggs, may occur as periphyton. An organism may remain ás periphyton throughout its life or during some period of its life cycle. An animal may be an obligatory or a facultative periphyton.

Term **biotecton** is preferred over **periphyton** these days, latter being a gramatically incorrect expression. On the basis of life forms biotecton are classified into bacteriotecton (bacteria), phytotecton (plants), and zootecton (animals). On the basis of substratum quality these are further categorized as: epiphytic (plant), epizoic (animal), epixylic (wood), epilithic (stone), epimetallic (metal), and epihalotic (other).

At times, the algal periphyton are so emmensely grown on macrophytes or as mats on the rocks in littoral zone that their role as primary producers in a system cannot be overlooked. Animal periphyton are preyed upon by animals of higher trophic levels and are, therefore, important in aquatic food chains. Some of the periphyton have been recognized as good indicators of water pollution.

1. Collection and preservation of periphyton

For microscopic periphyton, scrape carefully a known area (cm^2) and transfer the scrapping to a vial containing distilled water. Record the area scraped and nature of substratum. Preserve the sample with Lugol's iodine solution and formaldehyde as described for phytoplankton (see 3.3.2-1). For 10 ml of sample 2 drops of Lugol's iodine solution are enough. Allow the sample to settle and remove the supernatant if further concentration is desired.

Macroscopic periphyton are collected from the known area with the help of brush and forceps and are preserved in 4% formaldehyde (or 70% alcohol).

Before putting in a preservative the larger animals are anaesthetized and killed. For anaesthetization and killing strew menthol crystals or add drop by drop 70% alcohol on animal kept in water. Sponges may be killed by plunging them into 1% solution of osmic acid. Wash them with water before preservation. For the preservation of animals with calcareous structures (viz., molluscs, sponges etc.)70% alcohol should be used in place of formaldehyde. While preserving in alcohol delicate and soft bodied animals should be first put into 30% and 50% alcohols for several hours and finally into 70% alcohol to avoide shrinkage.

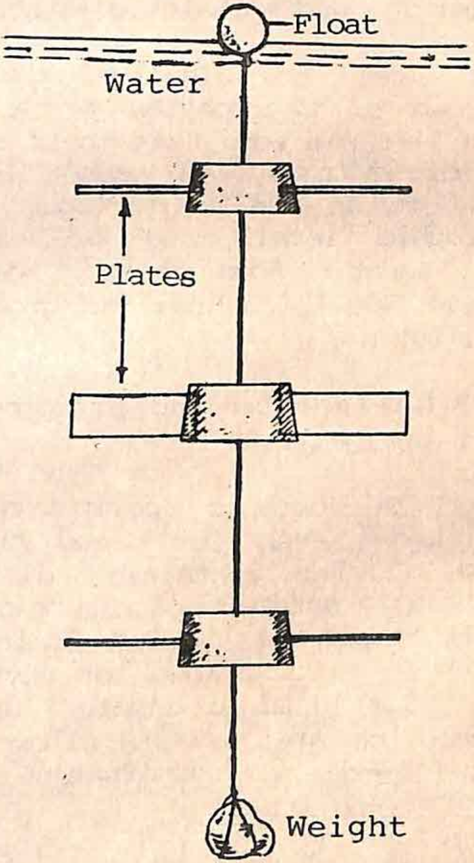

Fig.17. Periphyton sampler.

Plates of different material (such as glass, metal, plastic, slate etc.) are suspended in horizontal and vertical orders at various depths, as shown in Fig.17, and incubated for about 1 month. After and within the incubation period the plates are taken out and periphyton gathered on them are collected from a known area as stated above. Study of these organisms may reveal interesting information about colonization and substrate preference among periphyton of the body of water under investigation.

2. Qualitative analysis of periphyton

Identify the periphyton under microscope using pertinent literature. Among plants, various algae commonly occur as periphyton. Among animals, sponges, coelenterates, turbellarians, molluscs, and the eggs and larval stages of various animals may occur in periphytonic communities.

3. Measurement of periphyton population

Measure the periphyton population following any one of the two methods described for the enumeration of phytoplankton (see 3.3.2-3). Calculate the population per cm^2 as follows:

$$\text{Periphyton (units or organisms/cm}^2\text{)} = \frac{N \times C}{A}$$

Where, N = number of units or animals counted in 1 ml of sample (concentrate); C = total volume of sample (concentrate) in ml; and A = area scraped on the substratum (cm^2).

4. Measurement of periphyton biomass

To estimate the biomass of algal periphyton follow the method employed for phytoplankton biomass (see 3.3.2-4) and for animals method employed for zooplankton biomass (see 3.3.3-4). Express the results in g/cm^2

3.3.5. Benthos (Zoobenthos)

The heterogeneous assemblage of organisms attached or resting on the bottom or living in the bottom sediments of a body of water are known as benthos. **Phytobenthos** and **zoobenthos** are the terms used for benthic plants and animals respectively. In deeper waters the benthic communities are mainly represented by zoobenthos, besides microconsumers (bacteria and fungi). Phytobenthos are not so common in such a bottom due to adverse light conditions. The zoobenthos are represented by diverse animal phyla and include mainly the detritivores who constitute the primary consumer level in a detritus-type of food chain (as distinct from a grazing-type of food chain). On the basis of size they are classified into macrobenthos ($\geq$ 6 mm) and microbenthos ($<$ 6 mm).

The detritivore benthos are important in an aquatic ecosystem as they are capable of converting low quality detritus into high quality food that may be consumed at higher trophic levels. Benthic species and communities are considered to be the best indicators of organic pollution because of their constant presence, relatively long lives, sedentary habits, and differing tolerances to stress.

1. Collection, handling, and preservation of benthos

Benthos may be collected by using one of the many devices, such as scoops, grabs, dredges, and core samplers etc., depending upon the nature of the bottom and precision desired. Some common bottom samplers are as described below.

I. Scoop sampler (Fig.18): It is a very simple type of bottom sampler made up of a metallic conical cup with sharp cutting edge. The cup has a metallic strip towards its open face, to which a strong rope may be tied. The sampler is lowered to the bottom, dragged on the bottom sediments for some distance, and taken out of water. The sediment collected in the cup is processed as discussed later. This sampler is useful only for qualitative investigations, moreover some of the sample may wash away while drawing the sampler out.

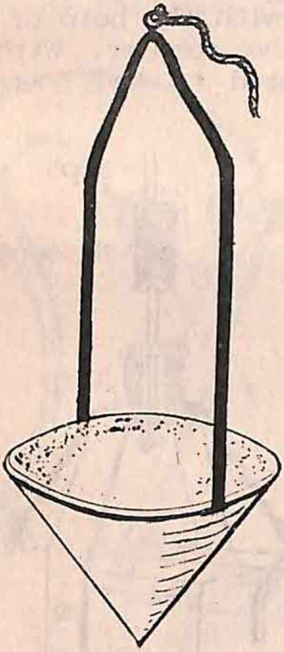

Fig.18. Scoop sampler.

II. Core sampler: The core sampler is made up of a piece of metallic pipe with its one end having sharp cutting edge. The sampler is pushed down vertically in the bottom sediment through its cutting edge, and taken out. The column of sediment passed into it is pushed out with the help of a piston and processed as discussed later. This sampler is very useful to study the profiles of a well settled sediment, because the sample obtained by it remains almost in unaltered stratified form.

III. Ekman-Birge grab (Fig.19): It is a convenient and widely used bottom sampler. It consists of a metallic box made up of two shovels closed together by a strong spring. These shovels can be kept open with the help of a trap provided at the top of the sampler. With a strong rope the grab is lowered to the bottom with its two

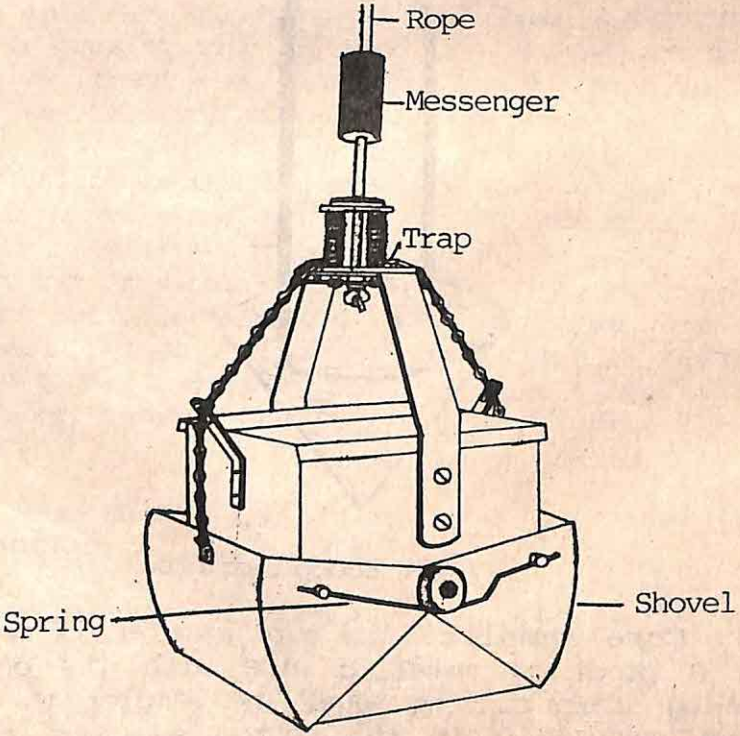

Fig.19. Ekman-Birge grab.

shovels open. When it has sunk into the bottom sediment, a metallic messenger (Fig.2) is released along the rope which hits the trap to open, and spring pulls the two shovels of sampler close. The grab is taken out and the sediment sample so received in it is processed as discussed later. The sampler is very useful for muddy bottoms. With the knowledge of its open area and volume the sample may be quantified as m^2 or m^3.

IV. Van Veen's grab (Fig.20): Like Ekman-Birge grab it is very convenient bottom sampler and works almost on the same principle. The two

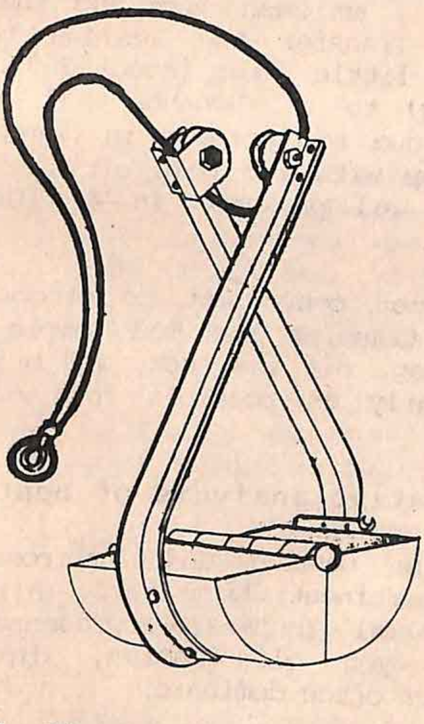

Fig.20. Van Veen's grab.

shovels of it are held open by a small bar and not by a trap. When the grab reaches the bottom the bar is released automatically. The draw rope is attached in such a way that with the pull from above, the two shovels of the sampler are closed tightly with mud sample captured in it. The sample is handled and preserved as discussed below. The advantage of this grab is that it does not require any messenger and is easy to operate. The area of the open grab is measured to calculate the area of sediment sampled by it.

Transfer the mud sample collected to a measuring cylinder or bucket and record its volume. The area of bottom sampled may be calculated by measuring the area of open shovels of grab. Add water to prepare a suspension and filter it first through a sieve of 2 mm mesh size and then of 0.5 mm mesh size. Transfer the residue to an enamel tray, add a little water (about 250 ml) and sugar (about 10 g) to it. Benthos will come to float on surface due to increase in density of water. Pick them up with the help of a dropper, brush, or forceps and preserve in 4% formaldehyde or 70% alcohol.

If it is not convenient to process the sample in field, transfer the mud sample to a strong polythene bag, tie its neck, and bring to laboratory as early as possible for sorting out the benthos.

2. Qualitative analysis of benthos

Identify the benthos under microscope with the help of pertinent literature. Although a wide array of animal groups are represented in a benthic community, yet oligochaetes, dipteran larvae, and molluscs often dominate.

3. Measurement of benthic population

Count the benthos species-wise under a binocular microscope, or by unaided eye if the animals are large enough, and calculate their population as follows:

$$\text{Benthos (organisms/m}^2\text{)} = \frac{N}{A} \times 10^4$$

Where, N = number of organisms per sample; and A= area of the sampler (cm^2).

4. Measurement of benthic biomass

The biomass of the benthos may be calculated by standard weighing techniue. Fresh and dry biomass (g/m^2), and ash content (%) etc. may be calculated on the lines suggested for macrophytes (see 3.3.1-3).

3.3.6. Saprotrophs (Bacteria and Fungi)

Saprotrophs, like auatic bacteria and fungi, are distributed throughout the water, but are especially abundant in the mud-water interface along bottom. A few saprotrophs are pathogenic causing disease among organisms, while most of them are harmless (or more aptly useful) and they cause breakdown (mineralization) of dead organic matter and release the nutrients for reuse in the material cycle of the system. The presence of large number of saprotrophs in water indicates the abundance of organic matter.

1. Bacteria: Standard plate count (SPC)

Srandard plate count is a measure of the density of aerobic and facultative aerobic bacteria.

Material

i. Laboratory glassware; ii. Petri plates; iii. autoclave; iv. spirit lamp; v. inoculation chamber; vi. incubator; vii. Quebec colony counter; and viii. chemicals, as given below:

A. Dilution water: (a). Dissolve 3.4 g of potassium dihydrogen phosphate in about 50 ml of distilled water. Adjust the pH to 7.2 by using 1N NaOH and further add distilled water to make the volume 100 ml. (b). Dissolve 3.8 g of magnesium chloride in distilled water to make the volume 100 ml. Take 1.25 ml of phosphate buffer solution (a) and 5.0 ml of magnesium chloride solution (b) in a volumetric flask and make up the contents 1 l by adding distilled water. Wait for 15-20 minutes and sterilize this dilution water in an autoclave.

B. Nutrient agar medium: Add 5.0 g of tryptone, 2.5 g of yeast extract, 1.0 g of glucose, and 15.0 g of agar to 1 l of distilled water. Sterilize the medium in an autoclave and adjust the pH to 7±0.1.

Method

Collect the sample in a sterilized bottle. Prepare dilutions of the sample with dilution water (chemical A) as follows:

Dilution	Dilution factor
1:0 (undiluted)	1.0
1:10	0.1
1:100	0.01
1:1000	0.001

Transfer 1 ml each of the above samples in separate sterilized Petri plates in at least three replicates of each. Pour about 12 ml of nutrient agar medium (chemical B) of about 45 °C temperature (heated in a water bath) to each of these Petri plates. Mix the medium and sample thoroughly by gently moving the Petri plates in circular order. When the medium is solidified, invert the plates and incubate at 37 °C for 48 hours in an incubator. On completion of incubation period count the bacterial colonies in each plate with the help of Quebec colony counter.

Calculation

$$\text{SPC (per ml)} = \frac{\text{Colonies counted}}{\text{Dilution factor}}$$

Take an average of SPCs for different sets (dilutions and replicates).

2. Fungi: Qualitative analysis

Material

i. Autoclave; ii. bait (help or mustard seeds); iii. Petri plates; iv. nutrient agar medium (see 3.3.6-1); v. inoculation chamber; vi. spirit lamp; and vii. incubator.

Method

Take 15 ml of undiluated sample water and sample water diluted with sterile distilled water aseptically in a set of Petri plates. Autoclave the bait (seeds) for about 15 minutes to sterilize and place 5 crushed baits to each Petri plate aseptically. Incubate the Petri plates at different temperatures. Examine periodically (daily or alternate day) for fungal growth. When good fungal growth is achieved, pick up some fungi and inoculate it aseptically in nutrient agar medium, in separate Petri plates, to grow further. Prepare subcultures from this fungi repeatedly to purify it and identify under microscope using pertinent literature. Phycomycetes are usually common in waters rich in decomposing organic matter, however, almost all fungal groups have been reported from aquatic environments.

3.3.7. Primary productivity

All heterotrophic life, directly or indirectly, depends for its energy requirements upon autotrophs who have unique capacity to trap the radiant energy of sun and transform it into high potential biochemical energy using inorganic matter (photosynthesis). Thus the primary productivity is defined as (i) the rate at which radiant energy is stored by photosynthetic and chemosynthetic activity of producer organisms (mainly green plants) in the form of organic matter which can be used as food; or (ii) the rate of increase in the biomass of primary producers; or (iii) simply the rate of photosynthesis. Gross primary productivity (GPP) is the total rate of photosynthesis, including the organic matter used up in respiration during the period of measurement; while net primary productivity (NPP) is the rate of storage of organic matter in plant tissue in excess of respiratory utilization by plants during the period of measurement. Thus gross production is the sum of net production and respiration.

The rate of primary productivity is influenced by a number of factors like light, temperature, nutrients, pollutants, and in and outflow of water etc. Eutrophic waters have a high rate of primary productivity because they harbour a high phytoplankton population in their trophogenic zone. On the basis of primary productivity the bodies of water are classified as:

Trophic status	Annual primary production (g C/m^2/year)
Oligotrophic	$<$ 100
Mesotrophic	100 - 200
Eutrophic	$>$ 200

In an aquatic ecosystem the process of primary productivity is accomplished by two main types of primary producers, macrophytes and phytoplankton. The measurement of macrophytic and phytoplanktonic productivities are discussed below.

1. Macrophytic productivity

Material

Same as that reuired for collection and quantitative analysis of macrophytes (see 3.3.1).

Method

Harvest the macrophytes randomly using quadrats (see 3.3.1-1). Repeat the harvesting from same locality after a definite interval of time (a few days). Estimate the biomass (fresh and dry), ash content (%), and ashfree dry weight (organic matter) after each harvesting as discussed earlier (see 3.3.1-3). Calculate the net primary productivity of macrophytes, using these values, as follows.

Calculation

$$\text{NPP } (g/m^2/\text{day}) = \frac{Bf - Bi}{D}$$

Where, NPP = net primary productivity (in terms of fresh, dry, or ashfree biomass, depending upon the values in which term of biomass are used in formula); Bi = biomass measured initially (g/m^2); Bf = biomass measured after definite interval (g/m^2); and D = time interval between initial and final harvstings (days).

2. Phytoplankton productivity

C^{14} and light and dark bottle methods are commonly employed for measurement of primary productivity of phytoplankton, of which the latter method being economic and easy, is being used satisfactorily in most of the laboratories for years. Here only light and dark bottle method is being given.

Material

i. All material, including reagents, as required for dissolved oxygen estimation (see 3.2.5-I); ii. among BOD bottles, two are kept transparent (referred to as light) while one is blackened from outside by paint (this set of three bottles is required for a single stratum at single station, but if productivity at different depths and stations in the water is to be estimated, as many sets as the depths and stations in question will be required); iii. float; and iv. rope.

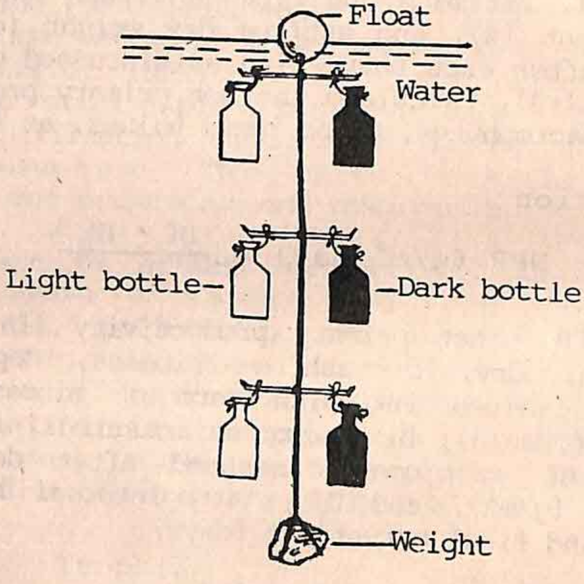

Fig.21. Light and dark bottles suspended for primary productivity estimation.

Method

Fill one set of three BOD bottles (2 light and 1 dark) with the sample of water from the depth in question, avoiding bubbling. Immediately determine the oxygen content (in mg/l) in one of the light bottles, as described earlier (3.2.5.-I). Suspend a set of one light and one dark bottle at the depth from which the sample was taken (Fig.21), and let them incubate for 24 hours (1 day). On completion of incubation period, take out the bottles and determine oxygen in each (in mg/l). Some workers prefer 12 hours' day.

Calculation

$$GPP = \frac{(LB - DB) \times 0.375}{T}$$

$$NPP = \frac{(LB - IB) \times 0.375}{T}$$

$$R = \frac{(IB - DB) \times 0.375}{T}$$

Where, GPP = gross primary productivity (gC/m^3/day or mgC/l/day); NPP = net primary productivity (gC/m^3/day or mgC/l/day); R = respiration (gC/m^3/day or mgC/l/day); IB = oxygen initially determined in light bottle (mg/l); DB = oxygen determined in dark bottle after incubation (mg/l); LB = oxygen determine in light bottle after incubation (mg/l); and T = time period of incubation (day).

When value of **T** is taken in hours the results will be expressed as gC/m^3/hour (= mgC/l/hour).

To get the values of primary productivity per m^2, multiply the average per m^3 values with the mean depth (m) of the body of water.

3.3.8. Chlorophyll

Photosynthetic pigments present in the cells of green plants are the important apparatus responsible for trapping the radiant energy to bring about photosynthetic reactions. Chlorophyll **a** is considered to be the most important phytopigment because of its occurrence in all plants, therefore, many workers prefer to determine only this chlorophyll than various chlorophylls or total chlorophyll. The distribution of chlorophylls **a**, **b**, and **c** in some important algal groups is as follows: Cyanophyceae - only **a**; Chlorophyceae -**a**, **b**; and Bacillariophyceae -**a**,**c**. Phaeopigments are the degradation products of chlorophylls and they increase with the age of the plants.

Chlorophylls being essentially involved with photosynthesis, their concentration is considered to be a very good index of primary production.

1. Estimation of chlorophyll in macrophytes

Material

i. Chemical balance; ii. mortar and pestle; iii. centrifuge; iv. spectrophotometer; v. laboratory glassware; vi. magnesium carbonate; and vii. acetone (90%).

Method

Collect the macrophytes and take 1 g of its fresh green part. Homogenize the material in presence of 20 ml of 90% acetone and a pinch of magnesium carbonate using a mortar and pestle. Transfer the contents to a dark bottle, cap tightly and place it in refrigerator for 6 hours (this period should be constant for all the samples) to allow complete extraction of chlorophyll. Centrifuge the contents at 3000 rpm for about 15 minutes and decant the supernatant into a volumetric flask of 25 ml. Make up the volume of contents to 25 ml by further adding 90% acetone and record the optical density (OD) of the extract on a spectrophotometer at 665 nm and 645 nm.

Simultaneously dry 1 g fresh weight of the same material in an oven at 105 °C till constant weight is attained. Record the dry weight.

Calculation

$$\underset{(\text{ mg/g DW})}{\text{Chlorophyll } \mathbf{a}} = A \times \frac{V}{1} \times \frac{FW}{DW} \times 1000$$

$$\underset{(\text{ mg/g DW})}{\text{Chlorophyll } \mathbf{b}} = B \times \frac{V}{1} \times \frac{FW}{DW} \times 1000$$

Where, A = [11.6 (OD at 665 nm) - 1.3 (OD at 645 nm)]; B = [19.1 (OD at 645 nm) - 4.7 (OD at 665 nm)]; V = volume of extract (25 ml); FW = fresh weight (g); DW = dry weight; and 1 = path length of cuvette of spectrophotometer (cm).

2. Estimation of chlorophyll in phytoplankton

Material

i. Glass fibre filter (Whatman GF/C); ii. filteration unit with a vaccum pump; iii. centrifuge; iv. spectrophotometer; v. laboratory glassware; vi.magnesium carbonate; and vii. acetone (90%).

Method

Take 500 ml of sample (or more or less depending upon the richness of algae in it as evident from the greenness of water) and add a little aqueous suspension of magnesium carbonate so as to avoid the degradation of chlorophyll and formation of phaeopigments. Filter the sample through glass fibre filter in a filteration unit. On completion of filteration remove the glass fibre filter from the unit and put into a tight capped dark glass bottle containing 10 ml of 90% acetone. Place it in refrigerator for 6 hours (this time should be constant for all the samples) for complete extraction of phytopigments. Then take out the extract and centrifuge it at 3000 rpm for about 15 minutes. Transfer the supernatant to a volumetric flask of 10 ml and make up the volume of contents to 10 ml by further adding 90% acetone. Record the optical density (OD) of the extract on a spectrophotometer at 630 nm, 645 nm, 663 nm, 665 nm, and 750 nm.

Acidify the extract with 2-3 drops of 1% hydrochloric acid and again record the OD at 665 nm and 750 nm.

Calculation

$$\underset{(\mu g/l \text{ or } mg/m^3)}{\text{Chlorophyll } \mathbf{a}} = A \times \frac{V_2}{V_1 \times 1}$$

$$\underset{(\mu g/l \text{ or } mg/m^3)}{\text{Chlorophyll } \mathbf{b}} = B \times \frac{V_2}{V_1 \times 1}$$

$$\underset{(\mu g/l \text{ or } mg/m^3)}{\text{Chlorophyll } \mathbf{c}} = C \times \frac{V_2}{V_1 \times 1}$$

$$\underset{(\mu g/l \text{ or } mg/m^3)}{\text{Phaeopigments}} = 26.7 \times P \times \frac{V_2}{V_1 \times 1}$$

Where, A = [11.64 (OD at 663 nm) - 2.16 (OD at 645 nm) + 0.1 (OD at 630 nm)]; B = [20.97 (OD at 645 nm) - 3.94 (OD at 663 nm) - 3.66 (OD at 630 nm)]; C = [54.22 (OD at 630 nm) - 14.81 (OD at 645 nm) -5.53 (OD at 663 nm)]; P = [1.7(OD at 665 nm after acidification - OD at 750 nm after acidification) - (OD at 665 nm - OD at 750 nm)]; V_1 = volume of sample filtered (l); V_2 = volume of extract (ml); and 1 = path length of cuvette of spectrophotometer (cm).

3.3.9. Decomposition

Decomposition is the process of mineralization of organic matter brought about mainly by the biological agencies, like bacteria and fungi. Bacteria perhaps are more important in breakdown of animal matter while fungi in breakdown of plant tissue. The delicate matter, like dead phytoplankton, is decomposed at a much faster rate as compared to regid tissue, like wood. Microbial population and temperature are the important factors governing the rate of process. The long and complex process of degradation of organic matter controls many important functions in ecosystem, such as (i) recycling of nutrients through mineralization of dead organic matter; (ii) production of food for a sequence of organisms in detritus food chain; and (iii) modification of the inert material of the earth surface.

This makes it clear that decomposition is an absolutely vital function because, without it, all the nutrients would soon be trapped in dead bodies, and no new life could be produced. AS such, the rate of decomposition in a system is a scale of rate of nutrient release which controls system's metabolism.

Material

i. Litter bags, made up of nylon net (mesh size 1 to 10 mm, depending upon the size of body to be decomposed); and ii. all material used in quantitative analysis of macrophytes (see 3.3.1-3).

Method

Take a little amount of plant (like macrophyte) or animal tissue. Note its fresh weight (FW). Dry it in an oven at 105 °C till a constant weight is attained and record the dry weight (DW). Incinerate the dry sample at 550 °C for a day in a muffle furnace, cool, and note the ash weight (AW).

Take same amounts of fresh weight of the tissue in a number of litter bags and incubate the bags at the bottom of the body of water. With the process of decomposition the tissue is disintegrated and products are released in the surrounding water. After regular interval of a few days (7 or 10) take out a few bags and make the measurements of dry weight and ash weight (as stated above) of the leftover matter in the bags. Make the calculation for loss of organic metter due to decomposition per g organic matter per day as follows.

Calculation

$$OM = DW - AW$$

Where, OM = organic matter (g); DW = dry weight (g); and AW = ash weight (g).

$$\text{Decomposition rate (g/g OM/day)} = \frac{OM_2 - OM_1}{OM_i \times D} \times 1000$$

Where, OM_1 and OM_2 = organic matter noted on two different days (g); OM_i = organic matter noted initially (g); and D = time period between assessment of OM_1 and OM_2 (days).

3.3.10. Sedimentation

Sedimentation in water is the process of sinking of particulate matter, both organic and inorganic, from upper to lower strata and ultimately to the bottom due to gravitational force. The rate of sinking of a particle depends upon its density and size, and also upon the viscocity and turbulence of the water. The rate of sedimentation of organic matter gives an idea about rate of the downward movement of the carbon fixed in trophogenic zone of the system.

Material

i. Graduated cylinders (or sedimentation sampler); ii. chemical balance; iii. filter paper (Whatman, ashfree); iv. oven; v. crucible; and muffle furnace.

Method

Fill a graduated cylinder of known volume with water from the depth at which the rate of sedimentation is to be determine. Filter this sample through a pre-weighed (W_1) filter paper (Whatman, ashfree), dry the filter paper with residue in an oven at 105 °C till constant weight is attained and note the weight (W_2). Incinerate the filter paper in a crucible in muffle furnace at 550 °C for 6 hours, cool, and weigh the ash content (W_3) as a difference between weight of crucible with dried filter paper and curcible with ash.

Now suspend the cylinder in strictly vertical order, with its open end facing surface, at the depth from which the sample was taken. Draw it out carefully after 1 month. Following the above cited methods filter the contents through a pre-weighed (W_1') filter paper (Whatman, ashfree); dry the paper with residue and record the weight (W_2'); and incinerate and record the ash weight (W_3').

Calculation

$$OM_1 = (W_2 - W_1) - W_3$$

$$OM_2 = (W_2' - W_1') - W_3'$$

Where, OM_1, OM_2 = weight of initial and final organic matter, respectively (g); W_1, W_1'= weight of filter paper used for initial and final measurements, respectively (g); W_2, W_2'= weight of filter paper with dried residue at initial and final measurements, respectively (g); W_3 . W_3'= weight of ash content at initial and final measurements, respectively (g).

$$\underset{(\mathrm{gOM/m^2/month})}{\text{Sedimentation rate}} = \frac{OM_2 - OM_1}{\pi r^2} \times 10^4$$

Where, π = a constant (3.142); and r = radius of cylinder (cm).

Chapter 4

SOIL / SEDIMENT SAMPLING

Soil consists of the weathered layer of the earth's crust with living organisms and products of their decay intermingled. An aquatic sediment is the soil submerged in water.

4.1. COLLECTION AND HANDLING OF SOIL SAMPLE

Sampling sites, depth, and method to be employed for collection of soil sample should be decided keeping in mind the purpose of study and parameter(s) in question. For general characterization of soil a few random samples from the study area to the depth of about 15 cm may suffice. Deeper soil samples may be needed for the study of soil profiles.

Surface soil samples may be collected using tools like spade or shovel. Soil sample from just near the root of a large tree should be avoided. Soils from near the foundations of civil constructions may not represent their natural make-up, therefore, should be avoided. For collection of soil from deeper profiles special borer samplers are used.

Soil samples so collected should be put in thick quality polythene bags and immediately brought to the laboratory. For parameters like pH, redox potential, nitrogen, and phosphorus etc., analysis should be performed as early as possible. For many other edaphic factors the sample may be stored after drying it at about 40 °C. The dried soil may be ground using mortar and pestle and sieved through a 2 mm mesh-sized sieve.

4.2. COLLECTION AND HANDLING OF AQUATIC SEDIMENTS

The aquatic sediments may be collected by employing special instruments, like scoop, grab, and core samplers. For details of such samplers and their operation see 3.3.5-1. Sediment samples are handled in the manner soil is handled (see 4.1).

Chapter 5

SOIL / SEDIMENT ANALYSIS

5.1. PHYSICAL PARAMETERS

5.1.1. Bulk density

The bulk density of the soil is defined as the dry weight of a unit volume of it, and it is expressed as g/cm^3. Normally the bulk density ranges from 1.1 to 1.5 g/cm^3 for medium to fine textured soil and from 1.2 to 1.65 g/cm^3 for coarse textured soil, but it is slight higher in case of alkaline saline soils. The soils with high bulk density are inhibitive to root penetration, and have low permeability and infiltration. The bulk density is inversely related to pore space of soil.

Material
i. Oven; ii. measuring cylinder; and iii. chemical balance.

Method
Dry the soil sample in an oven at 105 °C

until a constant weight is attained. Transfer a little dried soil to a measuring cylinder and note the volume. Record the weight of this volume of soil on a balance.

Calculation

$$\text{Bulk density } (g/cm^3) = \frac{\text{Weight of soil (g)}}{\text{Volume of soil } (cm^3)}$$

5.1.2. Specific gravity

The specific gravity of the soil is directly related to its bulk density and it may be used as an index of some aspects of soil quality as in case of bulk density.

Material

i. Oven; ii. glass bottles; and iii. chemical balance.

Method

Dry the soil in an oven at 105 °C until a constant weight is attained. Fill a pre-weighed glass bottle of known volume with dried soil and record its weight. Fill another pre-weighed glass bottle of same volume with distilled water and record its weight.

Calculation

$$\text{Specific gravity} = \frac{A_2 - A_1}{B_2 - B_1}$$

Where A_2= weight of bottle with soil; A_1= weight of empty bottle used for soil; B_2 = weight of bottle with distilled water; and B_1 = weight of empty bottle used for water.

5.1.3. Moisture content

The water in soil is not only important as a solvent and transporting agent but it maintains texture and compactness of soil and makes it habitable for plants and animals. The moisture in soil is gained mainly from infilteration of precipitated water and irrigation, and its content in soil at any time depends upon the water holding capacity of soil and drains through percolation, evaporation, and uptake by plants etc.

Material
i. Oven; and ii. chemical balance.

Method
Take a fresh homogenized sample of soil and weigh it. Now dry it in an oven at 105 °C until a constant weight is attained. Cool in a desiccator and record the final weight of sample.

Calculation

$$\text{Moisture content (\%)} = \frac{I - F}{I} \times 100$$

Where, I = initial weight of sample (g); and F = final weight of dried sample (g).

5.1.4. Water holding capacity

The water holding capacity of soil depends upon the physical and chemical nature of it. When the soil is absolutely saturated with water, so that water fills all the pores between the particles of soil and there is no air space (as in case of aquatic sediments), the soil is said to be at its maximum water holding capacity or saturation.

Material
i. Oven; ii. perforated circular soil boxes (5.6 cm diameter, 1.6 cm height, bottom perforated with holes of 0.75 mm diameter); iii. filter paper

(Whatman No.1); iv. Petri dish; and v. chemical balance.

Method

Dry the crushed soil sample in an oven at 105 °C. Place a filter paper (Whatman No.1) inside the perforated bottom of the circular soil box, weigh the box, and fill it with dried soil sample. Note the waight of box filled with dried soil. Place the box in Petri dish of 10 cm diameter, containing water, for about 12 hours, so that water enters the box and saturates the soil. Take the box out of water wipe it dry on the outside, and record its weight.

Calculation

$$\text{WHC (\%)} = \frac{(W_2 - W_1) - (W_1 - W_o)}{(W_1 - W_o)} \times 100$$

Where, WHC = water holding capacity; W_o = weight of empty box (g); W_1 = weight of box with dried soil (g); W_2 =weight of box with water saturated soil (g).

5.1.5. Texture

Depending upon their size, the mineral particles of the soil or sediment may be classified as clay, silt, sand, or gravel, as shown in Table 6. Wentworth's classification of coarser sediments, based upon size (diameter) of particles, also includes boulder (> 256 mm), cobble (64 - 256 mm), and pebble (4 - 64 mm). On the basis of relative percentage of sand, silt, and clay various textural names of the soil are given in Table 7. The texture of soil greatly influences the vegetation and the animal life.

Table 6. Mineral particles of the soil/sediment and their size.

Mineral particle	Diameter (mm)
Clay	< 0.002
Silt	0.002 - 0.020
Sand (fine)	0.020 - 0.200
Sand (coarse)	0.200 - 2.000
Gravel (fine)	2.000 - 5.000
Gravel (coarse)	> 5.000

Table 7. Soil classes and relative percentage of mineral particles.

Soil/Sediment	Relative % of mineral particles		
	Sand	Silt	Clay
Sandy	85-100	0-15	0-10
Loamy	70-90	0-30	0-15
Sandy loam	43-80	0-50	0-20
Loam	23-52	28-50	7-27
Silt loam	0-50	50-88	0-27
Silt	0-20	80-100	0-12
Sandy clay loam	45-80	0-28	20-35
Clay loam	20-45	15-53	27-40
Silty clay loam	0-20	40-73	27-40
Sandy clay	45-65	0-20	35-45
Silt clay	0-20	40-60	40-60
Clay	0-45	0-40	40-100

Material

i. Shaking machine; ii. motor dispersion unit (This consists of a 0.5 h.p. motor with a propeller shaft and double-bladed propeller of stainless steel. The base of the unit contains a 600 ml beaker with the baffle grid fitted snugly in it.); iii. sieve (made from 70 mesh brass or copper gauze); iv. sedimentation cylinder (40 cm high, about 6.5 cm in diameter, and graduated to contain 1.25 l); v. pipettes (to deliver 20 ml at 20 °C in 25 to 30 seconds. One such pipette should have its lower stem 39 cm long and a ring etched around it at a distance of 28 cm from lower tip. One other pipette should have its lower stem 32 cm long and a ring etched around it at a distance of 22 cm from the lower tip.); vi. beakers (one of 500 ml, marked on outside to indicate the height of 10 cm above bottom; and other of 800 ml); vii. rubber pestle (It consists of a rubber stopper of 25 mm height and 20 mm diameter having a 25 cm long glass rod fitted in the centre at narrower end.); viii. stirring paddle (It is made up of a brass disc of 6 cm diameter and 3 mm thickness, attached to a 50 cm long spindle. The disc has 8 holes in it, each of about 6 mm diameter.); ix. hot water bath; x. Buchner funnel; xi. filter paper (Whatman No. 50); xii. camel hair brush; and xiii. reagents, as given below:

A. Hydrogen peroxide (6%).

B. Hydrochloric acid (2N): Dilute 175 ml of concentrated hydrochloric acid with distilled water to 1 l.

C. Hydrochloric acid (0.2N): Take 100 ml of 2N hydrochloric acid (reagant B) and dilute with distilled water to 1 l.

D. Sodium hydroxide solution (1N): Dissole 40 g of sodium hydroxide in distilled water to make the volume 1 l.

Method

Take 25 g of air-dry soil in a beaker of 800 ml, add 50 ml of hydrogen peroxide (reagent A), and allow to stand overnight. Then heat the beaker on boiling water bath until the vigorous reaction is ceased. If the soil is extra rich in organic matter, further add 30 ml of hydrogen peroxide and heat the beaker on water bath for another 10 minutes. Cool the beaker, clean its sides with a rubber pestle and add 25 ml of 2N hydrochloric acid (reagent B). Dilute the contents with distilled water to about 250 ml and thoroughly rub the soil with rubber pestle. Wait for one hour and then test the solution with a blue litmus paper to ensure the presence of an excess of acid. Filter the contents through a Buchner funnel fitted with filter paper (Whatman No. 50). Wash the soil with 200 ml of 0.2N hydrochloric acid (reagent C) and then with excess of water until the filterate is almost neutral to litmus.

Transfer the soil from Buchner funnel to 600 ml beaker of dispersion unit. Clean the last traces of soil from filter paper and funnel with the help of a camel hair brush and rinsing with water into the same beaker. The volume of suspension should not exceed 250 ml. Add 10 ml of 1N sodium hydroxide solution (reagent D) and dip the propeller of dispersion unit into beaker. Set the propeller in motion and control the speed to have vigorous stirring without the loss from splashing. Continue the mechanical dispersion for about 15 minutes, then take out the propeller and baffle grid, and rinse the two into beaker.

Filter the suspension in a sedimentation cylinder through a 70 mesh sieve. Wash maximum possible material through the sieve with a stream of water until cylinder is about one-half full. Transfer sieve to a tray and dry for sand content estimation.

Cover the cylinder with a brass cap and shake it for 12-16 hours in a shaking machine for complete dispersion. Then stand the cylinder, rinse the brass cap into it and dilute the suspension with distilled water to 1.25 l. Record the temperature of the suspension, according to which the time of pipetting and decantation is determined as shown in Table 8.

Table 8. Time of sedimentation at different temperatures.

Temperature	First pipette sample	Second pipette sample		Fine sand decantation	
	Depth 28 cm	Depth 22 cm	Depth 28 cm	Depth 10 cm	
°C	Min.	Hr.	Hr.	Min.	Sec.
8	18¾	24½	–	6	40
9	18	23¾	–	6	30
10	17½	23	–	6	20
11	17	24¼	–	6	10
12	16½	21¾	–	6	0
13	16¼	21¼	–	5	50
14	15¾	20½	–	5	40
15	15¼	20	–	5	30
16	15	19½	24¾	5	20
17	14½	19	24¼	5	10
18	14¼	18½	23½	5	0
19	13¾	18	23	5	0
20	13½	17½	22½	4	48
21	13¼	17¼	22	4	40
22	13	16¾	21½	4	30
23	12½	–	21	4	30
24	12¼	–	20½	4	20
25	12	–	20	4	15
26	11¾	–	19½	4	10
27	11½	–	19	4	5
28	11¼	–	28½	4	0
29	11	–	18¼	3	55
30	10¾	–	17¾	3	50
31	10½	–	17½	3	45
32	10¼	–	17	3	40
33	10	–	16¾	3	35

Stir the suspension vigorously by up and down strokes of stirring paddle. Take out the paddle and note the time of commencement of sedimentation. On completion of sedimentation time for first pipetting (see Table 8) take 39 cm stemmed pipette, close the upper stem of it with the forefinger and introduce gently the lower stem into suspension so that 28 cm mark on the pipette corresponds with the surface of suspension. Fill the pipette to 20 ml by gentle suction and deliver the sample into a pre-weighed silica crucible. Evaporate the sample to dryness on a hot water bath, place the crucible in an oven at 105 °C for about 12 hours, cool in a desiccator and weigh. The increase in the weight (W_1) indicates the weight of oven-dry silt + clay.

Let the sedimentation cylinder stand until completion of sedimentation time for second pipetting (see Table 8). Take 32 cm stemmed pipette, close its upper stem with forefinger and introduce gently the lower stem into suspension so that 22 cm mark of the pipette corrosponds with the new surface. Because at higher temperature the pipetting time becomes inconveniently short, the second pipetting may be made by using a 39 cm stemmed pipette lowered to 28 cm mark after the time period indicated in Table 8. Fill the pipette to 20 ml by gentle suction, transfer the sample to pre-weighed silica crucible, evaporate and oven dry the sample at 105 °C, and record the increase in weight (W_2) which indicates the weight of oven-dry clay.

When the sandy residue on 70 mesh sieve has dried, rub it lightly until fine material ceases to pass through. Transfer the coarse sand left in sieve to a pre-weighed silica crucible, oven dry it at 105 °C and find the increase in weight (W_3) which corresponds to the weight of oven-dry coarse sand.

After second pipetting, remove the suspension gently by means of a siphon tube, leaving behind about 4 cm high undisturbed sediment at the bottom of the sedimentation cylinder. Bring this sediment into suspension by agitating with a stream of water and transfer the contents to a 500 ml beaker marked at 10 cm above the bottom. Add whatever fine material passed out of 70 mesh sieve by rubbing. Let the beaker stand for 15 minutes. Decant the supernatant with no or minimum disturbance to the sediment. Fill the beaker again with water to the 10 cm mark, stirr the sediment, wait for 15 minutes and again decant the supernatant. Record the temperature and find the corresponding time of decantation for fine sand from Table 8. Now fill the beaker again to 10 cm mark with water and ensure thorough mixing of sediment. Allow to stand, and on completion of period for decantation, as noted from Table 8, decant the supernatant as completely as possible, without losing any sediment. Again fill the beaker with water to 10 cm mark and repeat the exercise until supernatant becomes almost devoid of any suspended matter. Now transfer the residue in the beaker to a pre-weighed silica crucible, evaporate and oven dry the sample at 105 °C, cool in a desiccator and note the increase in weight (W_4) which corresponds to the weight of oven-dry fine sand.

Calculation

Per cent fractions in air-dry soil:

$$\text{Coarse sand (\%)} = W_3 \times 4$$

$$\text{Fine sand (\%)} = W_4 \times 4$$

$$\text{Silt (\%)} = (W_1 - W_2) \times 250$$

$$\text{Clay (\%)} = (W_2 \times 250) - 1.6$$

Where, W_1 = weight of first pipette sample (g); W_2 = weight of second pipette sample (g); W_3 =

weight of sandy residue obtained on 70 mesh sieve (g); and W_4 = weight of sandy residue obtained by decantation (g).

5.2. CHEMICAL PARAMETERS

Soil is a mixture of various inorganic and organic chemicals, and shows some significant chemical properties. The major inorganic constituents are the compounds of Al, Si, Ca, Mg, Fe, K, and Na, however, compounds of B, Mn, Cu, Zn, Mo, Co, I, and F also occur in minor quantities. These salts occur in the form of weak solution. Carbonates, sulphates, nitrates, and chlorides of Ca, Mg, K, and Na are usually found in abundance in such solutions. Humus is the main organic constituent of soil and it may contain chemicals like proteins, amino acids, purines, pyrimidines, sugars, fats, aromatic compounds, alcohols, waxes, and resins etc.

Factors like nature of parent matter through which water percolates, climatic conditions (rainfall, temperature etc.), plant and animal life, and human activity (agriculture, industry etc.) largly influence the chemical nature of soil. The chemical nature of aquatic sendiments, apart from above factors, is also influenced by the inputs from and drains to the overlying water.

The life in the soil, both plant and animal, is greatly influenced by chemical nature of the soil. Growth of terrestrial plants is largely a function of nutrients they derive from soil. Nitrogen and phosphorus, followed by potassium, calcium, sulphur, and magnesium, merit high as plant nutrients and because of their requirement in large quantity they are regarded as **macronutrients.** Iron, manganese, copper, zinc, boron, silicon, molybdenum, chlorine, vanadium, and cobalt though essential but are needed in very minute quantities by plant, so are called **micronutrients** or trace elements. The nutritive requirements for optimum growth of various plant species vary greatly, both qualitatively and quantitatively, therefore, no generalization can be drawn. As such, different chemical parameters hereunder delt are not discussed in this context. For a general description of these factors refer to Chapter 3 (3.2).

5.2.1. Hydrogen ion concentration (pH)

Take 10 g of air-dry soil/sediment and add 100 ml of distilled water to make a suspension of 1:10 w/v dilution. Determine the pH of suspension following the method described for water (see 3.2.1). Mention the dilution while reporting the result.

5.2.2. Electrical conductivity (EC)

Take 10 g of air-dry soil/sediment and add 100 ml of distilled water to prepare a suspension (1:10 w/v). Record the EC as described earlier (see 3.2.2). Mention the dilution while expressing the result.

5.2.3. Redox potential

Take 10 g of air-dry soil/sediment and add 100 ml of distilled water to make a suspension (1:10 w/v). Determine the redox potential of suspension as that of water (see 3.2.3). Mention the dilution while expressing the result.

5.2.4. Alkalinity

Take 10 g of air-dry soil/sediment and add 100 ml of distilled water to prepare a suspension (1:10 w/v). Filter it through a filter paper (Whatman

No 44) and determine the alkalinity of filterate following the method described for water (see 3.2.8). In a fraction of same soil/sediment sample estimate the miosture content as given earlier (see 5.1.3).

Calculation

$$\text{Alkalinity (mg/g)} = \frac{F}{10} \times \frac{V}{W} \times \frac{1}{(100 - M)}$$

Where, F = alkalinity of filterate (mg/l); V = total volume of suspension (ml); W = weight of soil/sediment used in suspension (g); and M = moisture content of soil/sediment (%).

5.2.5. Chloride

Take 10 g of soil/sediment and add 100 ml of distilled water to prepare a 1:10 w/v suspension. Filter it through a filter paper (Whatman No. 44) and estimate the chlorides in filterate following the method given for water (see 3.2.10). Analyse a fraction of same soil/sediment sample for moisture content as described earlier (see 5.1.3).

Calculation

$$\text{Chloride (mg/g)} = \frac{F}{10} \times \frac{V}{W} \times \frac{1}{(100 - M)}$$

Where, F = chloride estimated in filterate (mg/l); V = total volume of suspension (ml); W = weight of soil/sediment used in suspension (g); and M = moisture content of soil/sediment (%).

5.2.6. Sulphate

Take 10 g of air-dry soil/sediment and add 100 ml of distilled water to prepare a suspension (1:10 w/v). Filter it through a filter paper (Whatman No. 44) and determine the sulphate in it following the method described for water, preferably the gravimetric method (see 3.2.12-II). Estimate in a fraction of same soil/sediment sample the moisture content following the method given earlier (see 5.1.3).

Calculation

$$\text{Sulphate (mg/g)} = \frac{F}{10} \times \frac{V}{W} \times \frac{1}{(100 - M)}$$

Where, F = sulphate estimated in filterate (mg/l); V = total volume of suspension (ml); W = weight of soil/sediment used in suspension (g); and M = moisture content of soil/sediment (%).

5.2.7. Nitrogen (Kjeldahl nitrogen)

Kjeldahl nitrogen includes organic and ammonia forms of nitrogen and does not take into account the nitrate-nitrogen.

Material

i. Kjeldahl distillation assembly (Fig.22); ii. laboratory glassware; and iii. reagents, as given below:

A. Catalyst mixture: Mix 20 g of copper sulphate, 3 g of murcuric oxide, and 1 g of selenium powder, and grind. Mix 2 g of this mixture with 40 g of sodium sulphate to prepare the catalyst mixture.

B. Sulphuric acid (concentrated).

C. Sodium hydroxide solution (40%): Dissolve 40 g of sodium hydroxide in 100 ml of distilled water.

D. Zinc granules.

E. Boric acid cum indicator solution: see 3.2.20-1-II.

F. Hydrochloric acid (0.1N): Take 8.34 ml of concentrated hydrochloric acid (12N) and dilute with distilled water to prepare 100 ml of 1.0N HCl. Dilute this 100 ml of 1.0N HCl to 1 l by adding distilled water and get 0.1N HCl.

Method

Take 10 g of air-dry soil/sediment in a 300 ml round bottom flask and add 20 g of catalyst mixture (reagent A) and 35 ml of sulphuric acid (reagent B). Heat the contents from bottom of the flask for about 2 hours. Cool the contents (digest), add about 100 ml of distilled water, wait for about 5 minutes, and deliver the supernatant into 1 l distillation flask **A** of Kjeldahl distillation assembly (Fig.22). Wash the residue with a little of distilled water several times and transfer the supernatant each time to the same distillation flask. Add 100 ml of sodium hydroxide solution (reagent C) and a few granules of zinc (reagent D). Take 25 ml of boric acid cum indicator solution (reagent E) in a 500 ml Erlenmeyer flask **B** and place it below the condensor **C** of distilla-

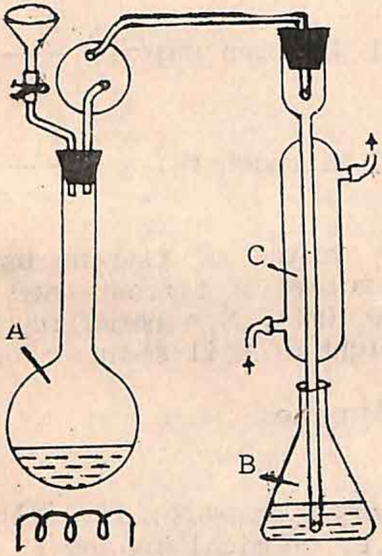

Fig.22. Kjeldahl distillation assembly.

tion assembly so that the lower open end of the condensor is dipped in solution. Heat the distillation flask on a hot plate and collect about 150 ml of distillate in flask **B.** Remove the flask with distillate and titrate the distillate (which has turned blue due to dissolution of ammonia) against 0.1N hydrochloric acid (reagent F). Turning of blue colour to light brown-pink indicates the end point. Run a distilled water blank in the same manner.

Calculation

$$\text{Kjeldahl Nitrogen (mg/g)} = \frac{T_1 - T_2 \times N \times 14}{W}$$

$$\text{Kjeldahl Nitrogen (\%)} = \frac{T_1 - T_2 \times N \times 1.4}{W}$$

Where, T_1 = volume of titrant used against sample (ml); T_2= volume of titrant used against distilled water blank (ml); N = normality of titrant (0.1); and W = weight of soil/sediment used (g).

5.2.8. Nitrate

Material

i. Laboratory glassware; ii. filter paper (Whatman No.50); iii. chemical balance; and iv. reagents (in addition to those used for nitrate estimation in water; see 3.2.20-3), as given below:

A. Extraction reagent: (a). Dissolve 12.5 g of copper sulphate in distilled water to make 100 ml of solution. (b). Dissolve 0.6 g of silver sulphate in distilled water to make 100 ml of solution. Mix 20 ml and 100 ml of solutions (a) and (b) respectively and dilute with distilled water to make 1 l of nitrate extraction reagent.

B. Calcium hydroxide: Dry, powdered.

C. Magnesium carbonate: Dry, powdered.

Method

Dry the soil/sediment in air and take 50 g of it in an Erlenmeyer flask (500 ml). Add 250 ml of extraction reagent (reagent A), shake for 15 minutes, add 0.4 g of calcium hydroxide (reagent B), shake for 5 minutes, and then add 1 g of magnesium carbonate (reagent C). Filter the contents through filter paper (Whatman No. 50) and measure the total volume of filterate. Determine the nitrate content in filterate following phenol disulphonic acid method described for water (see 3.2.20-3-I).

Calculation

$$NO_3\text{-N (mg/g)} = \frac{F \times V}{1000 \times W}$$

Where, F = NO_3-N determined in filterate (mg/l); V = total volume of filterate (ml); and W = weight of dried soil/sediment used (g).

5.2.9. Total phosphorus

Material

i. All material, including reagents, used in estimation of inorganic phosphorus in water (see 3.2.21-2); ii. filter paper (Whatman No. 44); iii. hot plate; and iv. other reagents, as given below:

A. Nitric acid (concentrated).

B. Perchloric acid (concentrated).

C. Sulphuric acid (diluted): Take 5 ml of concentrated sulphuric acid and dilute with distilled water to 100 ml.

Method

Air dry the soil/sediment, grind to fine powder, and take 0.5 g of it in a round bottom flask. Add a few drops of distilled water, 2 ml of nitric acid (reagent A), and 2 ml of perchloric acid (reagent B). Heat gently on a hot plate to dryness.

Cool, add 21 ml of sulphuric acid (reagent C) and boil for 15 minutes. Cool the flask, filter the digest through a filter paper (Whatman No. 44) and make up the volume of it to 250 ml with distilled water in a volumetric flask. Determine the phosphate content in solution following the mathod described for estimation of inorganic phosphorus in water (see 3.2.21-2).

Calculation

$$\text{Total phosphorus (mg/g)} = \frac{\text{Pd} \times \text{V}}{1000 \times \text{W}}$$

Where, Pd = PO_4-P in digest (mg/l); V = total volume of solution (ml); and W = weight of air-dry soil/sediment taken (g).

5.2.10. Phosphate

Matetial

i. All material, including reagents, required in estimation of inorganic phosphorus in water (see 3.2.21-2); ii. filter paper (Whatman No. 50); and iii. other reagent, as given below:

A. Sulphuric acid (0.002N): Dilute 2.8 ml of concentrated sulphuric acid to 1 l by adding distilled water. Take 20 ml of it and dilute to 1 l with distilled water to get 0.002N sulphuric acid.

Method

Air dry the soil/sediment sample and take 1 g of it in a 500 ml flask. Add 200 ml of sulphuric acid (0.002N) to it and shake for about half an hour. Filter the suspension through a filter paper (Whatman No. 50). Determine the phosphate content in the filterate following the method described for inorganic phosphorus in water (see 3.2.21-2).

Calculation

$$PO_4\text{-}P = (mg/l) = \frac{PS \times V}{1000 \times W}$$

Where, Ps = PO_4-P estimated in suspension (mg/l); V= total volume of suspension (ml); and W = weight of air-dry soil/sediment taken (g).

5.2.11. Calcium

Material

i. All material, including reagents, required for estimation of calcium in water (see 3.2.15); ii. filter paper (Whatman No.50); and iii. other reagents, as given below:

A. Ethyl alcohol: Absolute and 40%.

B. Ammonium acetate solution (1N): Take 57 ml of glacial acetic acid and dilute it to 800 ml with distilled water. Add gradually the concentrated ammonium hydroxide solution till pH 7.0 is attained. Further add distilled water to make the final volume 1 l.

Method

Preparation of soil extract: Air dry the soil/sediment and take 50 g of it in a flask. Add 100 ml of 40% of ethyl alcohol (reagent A), shake well, wait for about 10 minutes, and filter the suspension through filter paper (Whatment No. 50). Further wash the soil residue on filter paper with 40% ethyl alcohol and finally with absolute ethyl alcohol. Transfer the residue to a beaker, add 100 ml of ammonium acetate solution (reagent B), stir, and allow to stand overnight. Filter the supernatant through filter paper (Whatman No. 50) and collect the filterate (soil extract). Note the total volume of soil extract.

Determination of calcium: Determine the calcium in soil extract following the method described for water (see 3.2.15).

Calculation

$$\text{Calcium (mg/g)} = \frac{T \times 400.4 \times V_1}{V_2 \times W \times 10000}$$

Where, T = volume of EDTA titrant used (ml); V_1= total volume of soil extract (ml); V_2 = volume of soil extract titated (ml); and W = weight of air-dry soil/sediment taken for extaction (g).

5.2.12. Magnesium

Material

i. All material, including reagents, required for determination of magnesium in water (see 3.2.16); ii. filter paper (Whatman No. 50); and iii. other reagents, as given below:

A. Ethyl alcohol: Absolute and 40%.

B. Ammonium acetate solution: See 5.2.11.

Method

Prepare the soil extact as in case of determination of calcium (see 5.2.11). Find out magnesium content in the extact following the method given for determination of magnesium in water (see 3.2.16).

Calculation

$$\text{Magnesium (mg/g)} = \frac{T_2 - T_1 \times 400.4 \times V_1}{V \times 1.645 \times W \times 10000}$$

Where, T_2 = volume of EDTA titrant used for determination of calcium and magnesium (ml); T_1= volume of EDTA titrant used for determination of calcium (ml); V_1 = total volume of soil extract (ml); V_2 = volume of soil extract titrated (ml); and W = weight of air-dry soil/sediment taken for extraction (g).

5.2.13. Sodium

Material

i. All material, including reagents, required for determination of sodium in water (see 3.2.17); ii. filter paper (Whatman No. 50); and iii. other reagents, as given below:

A. Ethyl alcohol: Absolute and 40%.

B. Ammonium acetate solution: See 5.2.11.

Method

Prepare the soil extract as described for calcium (see 5.2.11). Determine the sodium content in extract following the method of sodium determination in water (see 3.2.17).

Calculation

$$\text{Sodium (mg/g)} = \frac{A \times V}{W \times 10000}$$

Where, A = sodium content of soil extract (mg/l); V = total volume of soil extract (ml); and W = weight of air-dry soil/sediment taken for extraction (g).

5.2.14. Potassium

Material

i. All material, including reagents, required for determination of potassium in water (see 3.2.18); ii. filter paper (Whatman No. 50); and iii. other reagents, as given below:

A. Ethyl alcohol: Absolute and 40%.

B. Ammonium acetate solution: See 5.2.11.

Method

Prepare the soil extract as described for determination of calcium (see 5.2.11). Find out the potassium in extract as it is determined in water (see 3.2.18).

Calculation

$$\text{Potassium (mg/g)} = \frac{A \times V}{W \times 10000}$$

Where, A = potassium content of soil extract (mg/l); V = total volume of soil extract (ml); and W = weight of air-dry soil/sediment taken for extraction (g).

5.2.15. Iron

Material

All material, including reagents, required for estimation of iron in water (see 3.2.19).

Method

Take 10 g of air-dry soil/sediment and add 100 ml of distilled water to make 1:10 w/v suspension. Filter it through a filter paper (Whatman No. 44) and determine the iron in filterate following the method used for iron estimation in water (see 3.3.19). Analyse a fraction of soil/sediment sample for moisture content as described earlier (see 5.1.3).

Calculation

$$\text{Iron (mg/g)} = \frac{F}{10} \times \frac{V}{W} \times \frac{1}{(100 - M)}$$

Where, F = iron determined in filterate (mg/l); V = total volume of suspension (ml); W = weight of soil/sediment used in suspension (g); and M = moisture content of soil/sediment (%).

5.2.16. Organic matter

Material

i. Laboratory glassware; and ii. reagents, as given below:

A. Potassium dichromate solution (1N): Dissolve 49.04 g of potassium dichromate in distilled water to prepare 1 l of solution.
B. Sulphuric acid (concentrated).
C. Phosphoric acid (concentrated).
D. Sodium fluoride: Dry, powdered.
E. Diphenylamine indicator: Dissolve 0.25 g of diphenylamine in 10 ml of distilled water and add gradually 50 ml of concentrated sulphuric acid.
F. Ferrous ammonium sulphate solution (0.5N): Add 20 ml of concentrated sulphuric acid to 800 ml of distilled water and dissolve in it 196.1 g of ferrous ammonium sulphate. Further add distilled water to make the volume 1 l.

Method

Air dry the soil/sediment and take 0.5 g of it in 500 ml Erlenmeyer flask. Add 10 ml of potassium dichromate solution (reagent A) and gradually 20 ml of sulphuric acid (reagent B). Wait for about half an hour, and then add 200 ml of distilled water, 10 ml of phosphoric acid (reagent C), 0.2 g of sodium fluoride (reagent D), and 1 ml of diphenylamine indicator (reagent E). Titrate the contents against ferrous ammonium sulphate solution (reagent F). At the end point the dull green colour changes through turbid blue to the brilliant green.

Run a distilled water blank simultaneously.

Calculation

$$\text{OM (mg/g)} = \frac{6.791}{W} \left[1 - \frac{T_1}{T_2} \right] \times 10$$

$$\text{OM (\%)} = \frac{6.791}{W} \left[1 - \frac{T_1}{T_2} \right]$$

$$\text{Carbon (\%)} = \frac{6.791}{W \times 1.724} \left[1 - \frac{T_1}{T_2} \right]$$

Where, OM = organic matter; W = weight of soil/ sediment taken (g); T_1 = volume of titrant used against sample (ml); and T_2 = volume of titrant used against distilled water blank (ml).

5.3. BIOLOGICAL PARAMETERS

Soil supports a wide array of plant and animal life. The biota may be categorized as one found on the surface (surface biota) and the other found within the soil (sub-surface biota). The qualitative analyses of flora and fauna can be made by using pertinent literature. The great diversity of terrestrial habitats renders it difficult to have a generalized approach for quantitative analysis of floral or faunal population, however, some common techniques are being discussed in this section.

5.3.1. Animal population density

I. Direct count method

This method is particularly applicable to the census of larger animals with lesser mobility. In this, the animals are counted in a known area and the result is expressed as number of animals per unit area.

II. Random sampling method

Fix the plots of known area randomly in a large locality and enumerate the animals in these plots. For highly mobile species larger areas need to be assessed while for animals with lesser mobility, like molluscs and earthworms, small quadrats may suffice. Take an average of count for various plots or quadrats and then calculate values per unit area.

III. Marking-recapture method

This method is very useful for enumeration of highly mobile animals, like birds, and the animals inhabiting hide-outs like burrows and caves. Capture a number of animals from a known area, count (precensus count), mark, and release them in the same area. Give them sufficient time to disperse in the environment. After this interval recapture the species from same region and count the total individuals and marked individuals caught. Calculate the population as follows:

$$\text{Animals/unit area} = \frac{N \times M}{m}$$

Where, M = number of individuals captured and marked in precensus period; N = number of total individuals recaptured; m = number of marked individuals recaptured.

5.3.2. Benthos

Benthos are the dwellers of aquatic sediments. Their population and biomass can be measured following the methods given earlier (see 3.3.5).

5.3.3. Plant population density

Mark the plots of known area randomly in the study area. Identify the plants in each plot using pertinent literature and count them species-wise. Calculate the population of each species per unit area from an average of counts in various plots.

5.3.4. Plant biomass

Harvest the plants from a known area and determine their biomass as described for macrophytes (see 3.3.1-3). Express the results as fresh and dry biomass per unit area.

5.3.5. Primary productivity

Estimate the primary productivity of a land ecosystem on the lines prescribed for macrophytic productivity estimation (see 3.3.7-1).

5.3.6. Bacteria: Standard plate count (SPC)

Take 1 g of fresh soil/sediment sample in a sterilized bottle. Add 10 ml of aseptic distilled water to prepare a suspension. Decant the supernatant in other sterilized bottle and use it as sample to make SPC which is a measure of the density of aerobic and facultative aerobic bacteria. Follow the method prescribed for making SPC of water (see 3.3.6-1). Multiply the SPC/ml thus calculated by 10 to express the result as SPC/g of soil.

5.3.7. Decomposition

The rate of decomposition can be determined by decomposing the samples of litter in fine-meshed litter bags made up of nylon, as described for measurement of decomposition rate in water (see 3.3.9), but in this case the bags are incubated in soil instead of water.

Section C

Chapter 6

AIR SAMPLING

The lowest layer of atmosphere that extends to an altitude of 11 km is called **trophosphere.** The gaseous mixture of trophosphere is called air which is relatively constant in composition and essential for most of the organisms. Trophosphere is the region of strong air movements and cloud formation, hence it is regarded as the locus of most weather phenomena which affect different ecosystems of the biosphere.

Physical factors pertaining to air are measured in the field using special monitoring instruments. For chemical analysis of air two methods have been in use.

I. Grab sampling

In olden days, when the analytical techniques were not evolved to the present extent, the air samples were used to be collected in large vessels and brought to the laboratory for analysis. The technique is still in use for spot examinations. This mode of sampling has limitations like leakage in transport, contamination, and limited volume of air that can be analysed.

II. Continuous monitoring

Presently, continuous air monitoring systems based on field instruments are more popular because of their operational ease and precision of results. Such instruments are mainly based on the colorimetric (photometric), flame emission, conductimetric, and amperometric principles, of which the first principle is the most common for many instruments. The air to be analysed is pumped through a reaction chabmer where it reacts with the chemicals circulated in a close cycle. The products of reaction may then be determined photometrically or by other techniques mentioned above, and the calculations are made for the parameter in question from these values.

Air monitoring, for general purpose, should be made in open atmosphere away from the gaseous escape points. For monitoring of industrial and vehicular emissions into the air, the air should be analysed at the exhaust stack and pipe respectively.

Chapter 7

AIR ANALYSIS

7.1. PHYSICAL PARAMETERS

7.1.1. Wind velocity

The wind may be defined as the motion of air caused by gravity, deflective forces due to earth's rotation from west to east, and centrifugal force due to curvature of the wind path. These forces are altered by factors like friction and density.

The wind is an important ecological factor of atmosphere. It alters a many other environmental factors like temperature, light, water balance etc. and influences animal and plant life. Baufort's scale classifies the wind into 12 categories based on velocity and effects (Table 9).

The instrument used to measure wind velocity is called anemometer. Of various types of anemometers in use, rotational (cup and propeller type) and pressure-plate types are more common.

Table 9. Wind types based on velocity and effects. Figures in parantheses denote mean values.

Baufort's scale	Wind type	Wind velocity (km/hr)	Effects
0	Calm	<1	Smoke rises up.
1	Light air	1-5 (3)	Smoke drifts slowly in wind direction.
2	Light breeze	6-11 (9)	Wind felt on face, leaves rustle.
3	Gentle breeze	12-19 (16)	Leaves and small twigs in constant motion, light flag extended.
4	Fresh breeze	20-28 (24)	Small branches move, raises dust.
5	Moderate breeze	29-38 (34)	Small trees sway.
6	Strong breeze	39-49 (44)	Large branches in motion, whistling of telegraph wires.
7	Near gale	50-61 (55)	Whole tree in motion, difficulty in walking.
8	Gale	62-74 (68)	Breaks twigs of trees.
9	Strong gale	75-88 (82)	Some damage to vegetation and civil structures.
10	Storm	89-102 (96)	Trees uprooted, considerable structural damage.
11	Violent storm	103-117 (110)	Widespread destruction.
12	Hurricane	> 117	Total destruction.

I. Rotational type anemometer

The cup anemometer (Fig.23) is the most common rotational type anemometer largely used by meteorologists. The instrument is mounted in an elevated situation where wind causes rotation of its cups and the movement of wind over a selected period of time is recorded from the meter provided near the foundation. The wind velocity is then calculated in m/second or km/hour.

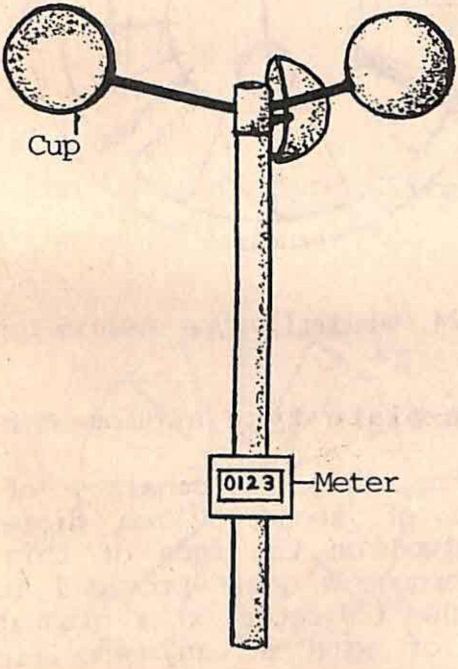

Fig.23. Cup type anemometer.

The other rotational type anemometer is of propeller or windmill type (Fig.24). It is a portable instrument provided with a wheel of flat or helicoidal blades like that of a windmill which rorates by the action of wind. The wind movement over a selected period of time is recorded on the dial and the velocity is calculated in m/second or km/hour.

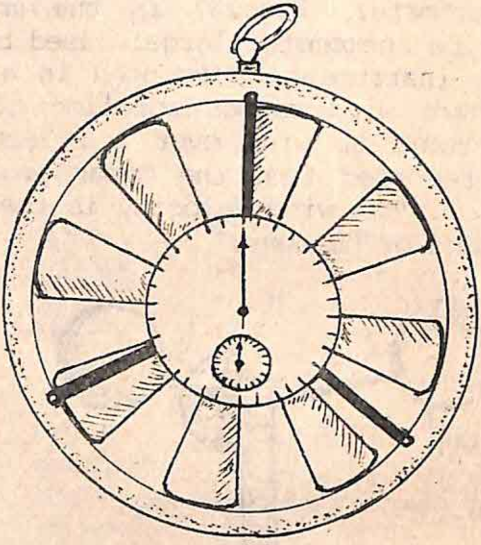

Fig.24. Windmill type anemometer.

II. Pressure-plate type anemometer

This anemometer (Fig.25) consists of a round pressure-plate of about 30 cm diameter. Wind pressure received on the face of this plate is transmitted through a shaft provided at its back which moves the indicator on a graduated scale. The velocity of wind at any specific time is read from the scale.

The direction of wind is also an important feature which is noted from the wind direction recorder fitted with most of the anemometers. This recorder is initially set in the appropriate directions by using a magnetic compass. The direction is expressed in 16 point scale of geographical directions (Fig.26).

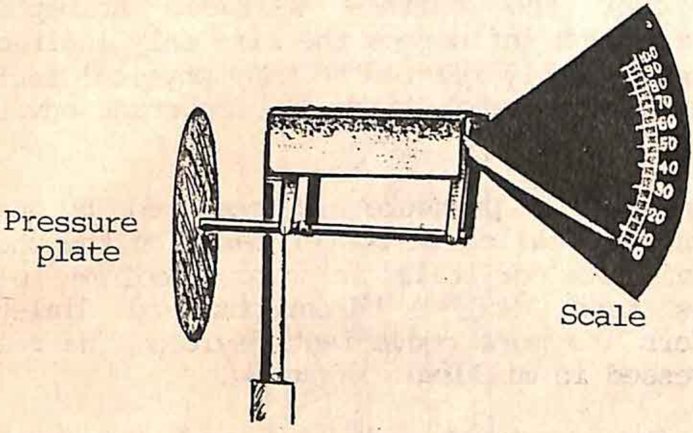

Fig.25. Pressure-plate type anemometer.

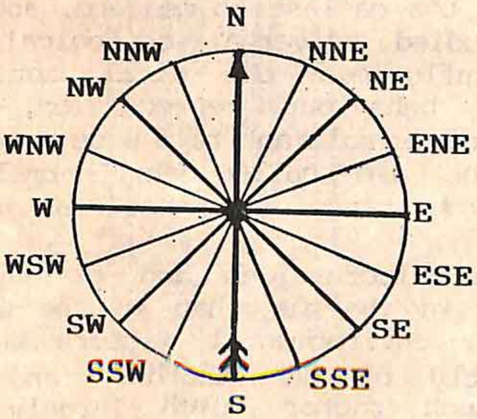

Fig.26. Sixteen point scale of geographical directions.

7.1.2. Atmospheric pressure

At MSL, atmospheric pressure is 1013.25 millibars (= 760 mm Hg). It decreases with the rise in altitude, and is also influenced by the temperature gradients. Development of low and high pressure cells in the atmosphere causes movement of air masses over the earth's surface. Atmospheric pressure though influences the life only indirectly, yet being directly related to many physical factors (weather and climate) it is an important environmental parameter.

The atmospheric pressure is measured by using an instrument called barometer based on the phenomenon of rise or fall in murcury column along a glass tube. Modern barographs and dial-type barometers are more convenient devices. The result is expressed in millibars of mm Hg.

7.1.3. Temperature: minimum and maximum

Temperature is a measure of intensity of heat in terms of a standardized unit. It is an extremely important, the easiest to measure, and most extensively studied universal ecological factor. It greatly influences the vital activities like metabolism, behaviour, reproduction, and development. Organisms tolerant to a wide range of thermal fluctuations are called eurythermal and those to a narrow range are known as stenothermal.

Temperature fluctuations and extremes are more pronounced in the air than in the water medium. In nature, environmental factors do not operate independently of one another, and temperature is one such factor which largely influences, and is influenced by, other factors like moisture and wind. Diurnal and annual ranges of atmospheric temperature are more important in any ecological investigation rather than single time temperature recording.

Material

A minimum-maximum thermometer (Fig.27): It is a

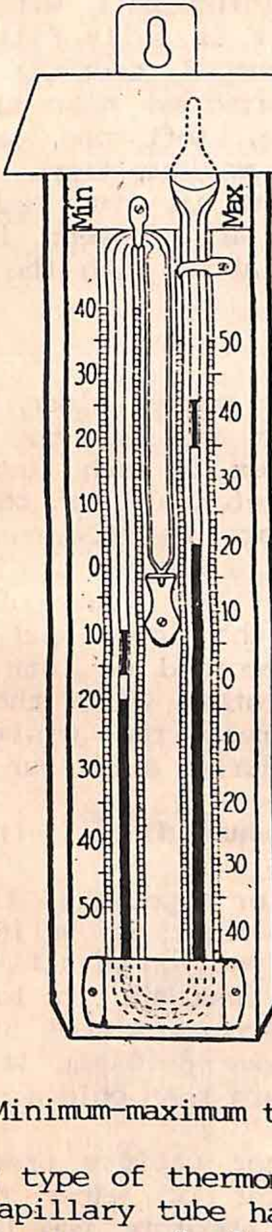

Fig.27. Minimum-maximum thermometer.

self registering type of thermometer. It consists of a U-shaped capillary tube having an elongated reservoir at the upper end of left limb and pear shaped reservoir on the top of right limb. The lower half of the U-shaped tube is filled with murcury and remaining part with creosote. Left elongated reservoir is fully filled with creosote while right pear-shaped reservoir partly. Temperature scales are provided near the two limbs of the U-shaped tube, left one reads the minimum and right one the maximum temperature. Just above the murcury column in two limbs fine indices made up of iron are present in the capillary tube which move about with the rise in murcury level.

Method

Bring a horse-shoe magnet close to the capillary tube of thermometer and with the downward strokes of it set the index in each limb to rest on the murcury column. Preferably set the indices during morning hours. Place the thermometer in a shady atmosphere. After a lapse of 24 hours record the temperature in two limbs of the instrument as indicated by the lower end of each index. The temperature recorded on left limb represents the minimum temperature while that on right limb the maximum. Express the minimum and maximum temperature in °C during a 24 hour diel cycle.

7.1.4. **Relative humidity** (RH)

The content of water vapours in the air is called humidity and by weight it varies from 0.02 to 4%. Humidity is a function of altitude and temperature. It is most abundant in lower profiles of atmosphere, but becomes scarce as one goes high and almost nil above 8-10 km. Warm air contains more of water vapours than cold air.

The amount of water vapours present in the air expressed as weight of water per unit weight of air is called **absolute humidity**. Because the amount of water vapour air can hold at saturation varies with temperaturre and pressure, **relative humidity** represents the percentage of water vapour actually present compared with what would be expected at saturation under existing conditions of temperature and pressure.

Relative humidity is an important ecological measurement. It shows a diel rhythm and marked vertical and horizontal gradients. Humidity, together with other physical factors like temperature and light, plays an important role in regulation of activities and distribution of organisms. It is especially important environmentally as it modifies the effects of temperature. The rate of evapotranspiration from the surface of organisms is much influenced by ambient humidity.

Although dial-type hygrometers, hygrographs, and electrical sensing devices to measure the RH are available yet conventional psychrometer consisting of wet and dry bulb thermometers is widely used for this purpose.

Material

A psychrometer (Fig.28): This instrument consists

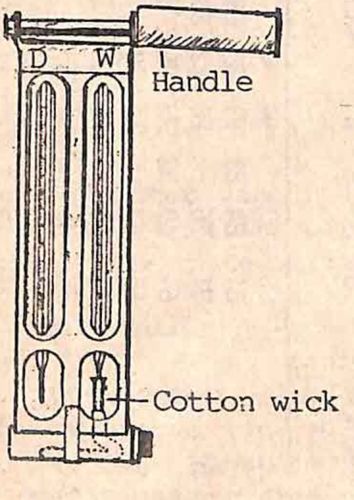

Fig. 28. Psychrometer.

of two ordinary thermometers marked as wet-bulb (W) and dry-bulb (D) thermometers. The bulb of wet-bulb thermometer is kept saturated with water by covering it with a cottom wick dipped in the water.

Method

Soak the cotton wick of wet-bulb thermometer in water and swing the set of thermometers (psychrometer) in air with the help of a handle provided at the top of the instrument, until the readings in two thermometers are constsnt. Record the temperature in both the thermometers and find the difference in two readings referred to as depression in wet-bulb thermometer. Find the RH (%) following Table 10 which involves temperature in dry-bulb thermometer and depression in temperature in wet-bulb thermometer. If the two thermometers read same temperature then this is indicative of 100% RH.

7.2. CHEMICAL PARAMETERS

The chemical composition of trophosphere (the lowest layer of atmosphere upto an altitude of 11 km) at sea level, excepting water vapours and air-borne particles, is given in Table 11. Nitrogen, oxygen, and argon constitute the major part (99.97%) of total volume while carbon dioxide accounts for almost all the remaining part (0.03%). Other gases, as enlisted in Table 11, are found in traces. The content of water vapour in trophosphere ranges from 0.02 to 4.0% by weight while that of particulate matter is quite variable.

Since a variety of gas analysers are marketed today which are convenient to use and reliable too, the analytical procedures involving such instruments are not described in detail here and only a mention of principle involved is made.

Table 10. Relative humidity (%) as measured by a psychrometer.

Depression in wet-bulb thermometer (°C)	Temperature in dry-bulb thermometer (°C)																			
	0	3	5	6	9	10	12	15	18	20	21	24	25	27	30	33	35	36	39	40
0.5	90	92		94	94		94	95		96		96		96	96	96		97	97	
1	81	84	87	87	88	88	89	90	90	90	91	92	92	93	93	93	93	93	94	94
1.5	71	76		80	82	83	84	85	86		87	88		90	90	90		90	91	
2	64	69	72	73	76	76	78	80	82	82	83	85	85	86	86	86	87	87	88	88
2.5	55	62		66	70		73	76	78		79	81		82	82	83		84	85	
3	46	54		60	65		68	71	73		75	77		79	79	80		81	85	
3.5	38	46		54	59		63	66	69		71	74		76	76	77		78	79	
4	29	40		47	53		58	62	65		67	70		72	73	74		75	76	
4.5	21	32		41	48		53	58	61		64	66		68	70	71		72	74	
5	13	25	33	35	42		48	53	57	59	60	63	63	65	67	68	70	70	71	72
6		12	21	23	32	34	38	44	52	52	53	56	57	59	61	63	64	64	66	66
7			9	11	22	25	30	36	42	45	46	49	50	53	55	57	59	59	61	61
8					12	15	21	28	35	38	39	43	44	47	50	52	54	54	56	56
9					3	6	12	20	27	30	32	37	38	41	44	47	50	50	52	52
10							4	13	20	24	26	31	33	36	39	42	44	45	47	48
11								4	13		19	26		31	35	37		41	43	
12									6		13	21		26	30	33		36	39	

Table 11. Chemical composition of a dry standard atmosphere at sea level.

Gas	Percentage
Nitrogen	78.09
Oxygen	20.95
Argon	0.93
Carbon dioxide	0.03
Neon	1.8×10^{-3}
Helium	5.24×10^{-4}
Krypton	1.0×10^{-4}
Hydrogen	5.0×10^{-5}
Xenon	8.0×10^{-6}
Ozone	1.0×10^{-6}
Radon	6×10^{-18}

7.2.1. Particulate matter

The particulate matter in air may occur as solid and liquid both. The common solid forms are dust, fumes, and smoke while common liquid forms are fog and mist. Particles larger than a molecule but small enough to remain dispersed in air are also called **aerosols**. The particulate matter in air is a variable component and is introduced through natural phenomenon like wind, volcanic emanations, pollen and spores, decomposition of organic matter etc., or through human activities like industrial processes, mining, burning of fuel etc.

Air-borne particles are considered to be a nuisance to industries requiring clean and aseptic atmosphere like drug and food industry. They may cause diseases like allergic asthma, bronchitis, emphysema, and even fibrosis of lungs. Certain green plants are found to be good dust collectors rendering the air clean.

I. Gravitational method

This method can be used to evaluate the dust fall within a city or an industrial shed, and usually it accounts for particles of 1 micron or larger size.

Put the clean glass jars of known open area in the atmosphere where dust fall is to be estimated. After a sufficient gap of time (some hours or days) collect the dust settled in each jar and weigh it. Take an average weight of dust in each jar and calculate the result of dust fall to be expressed as weight of dust per unit area per unit time.

II. Impaction method

An impactor device (Cascade impactor; Fig. 29) is based on the principle that particles are unable to follow the rapid directional change and are impacted on the surface to which they hit (collecting surface). Very small particles,

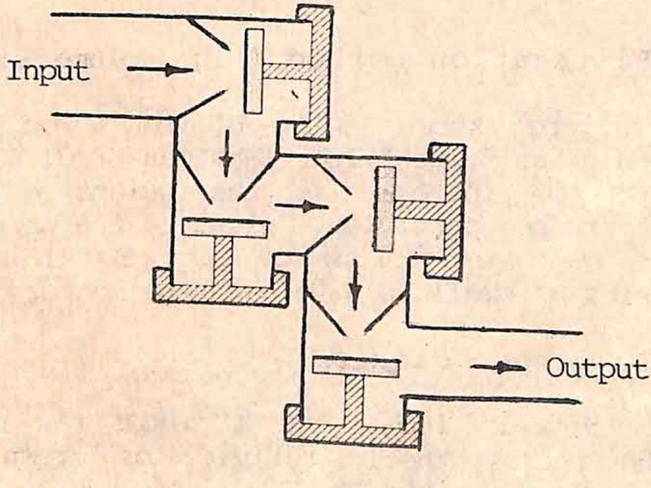

Fig.29. Cascade impactor.

however, are not collected in this inertia collector.

Draw a known volume of air through a slit or jet in an impactor device, using a suction pump. After a considerable volume of air has passed through, stop suction and weigh the particles collected in the device. The count of the particles can be made by observation through a microscope. Express the result as weight of dust or number of particles per unit volume (l or m^3) of air.

III. Electrostatic precipitation method

An electrostatic precipitator consists of an ionizing electrode charged with high negative potential (15,000 to 30,000 V, D.C.) and a collecting tube charged with positive potential produced by a special transformer and rectifier.

Pass a known volume of air through the precipitator where the incoming particles are negatively charged by the electrrode and are adhered to the positively charged collecting tube. Weigh the particles collected on a balance and count them under a microscope.

IV. Filteration method (High volume sampling)

Pass a large known volume of air through a preweighed glass fibre filter (Whatman GF/C) and then measure the increase in the weight of filter due to trapped particles. Calculate the particulate matter per unit volume of air. By this method particles as small as 0.05 µ can be measured.

7.2.2. Carbon dioxide

Carbon dioxide (CO_2) is a basic raw material in the photosynthetic activity of green plants without which hardly any life can subsist. Photosynthesis in many plants has been reported to have increased with the moderate increase in CO_2 concentration. A high CO_2 content in air, however, impairs normal respiratory activity of oganisms.

The major reservoir pool of biospheric CO_2 lies in carbonate system of the oceans. Respiratory activity of organisms together with decomposition accelerated by man's activities like burning of fuel and agricultural practices also add to the atmospheric CO_2. Although the CO_2 injected into air by man's agroindustrial activities is quite small as compared to the total CO_2 in circulation yet it is begining to have perceptible effect because the atmospheric reservoir is small and the larger oceanic reservoir is not able to absorb the CO_2 at the rate it is being produced by man. Climatologists are of view that even small changes in atmospheric CO_2 can have major effects on climate. The measurement of CO_2 changes in terrestrial environment is, therefore, considered to be more practical by ecologists rather than oxygen changes.

Material

i. Zecondroff apparatus (Fig.30); and ii. working solution of sodium carbonate: Boil and cool the distilled water and dissolve 0.53 g of sodium carbonate in it to make the volume 100 ml. Add 0.1 g of phenolphthalein to it and shake gently. Take 1 ml of this solution and add distilled water to make 100 ml of working solution.

Method

Take 10 ml of working solution of sodium carbonate in Zecondroff apparatus (Fig.30). Take the apparatus to a benign environment and pump the air through the bulb and shake after each stroke until the pink colour of solution disappears. Count the number of strokes of pump applied (B). Now wash the apparatus thoroughly. Put fresh

10 ml of working solution of sodium carbonate in it and take the apparatus to the atmosphere where CO_2 is to be monitored. Repeat the stroking of pump until the solution turns colourless, and count the number of strokes applied (S).

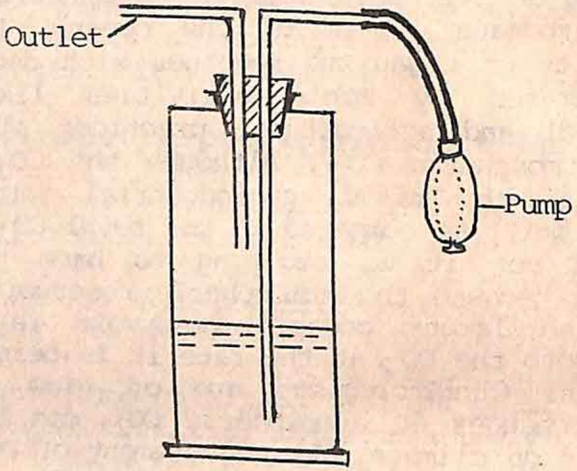

Fig.30. Zecondroff apparatus.

Calculation

$$CO_2\ (\%) = \frac{B}{S \times 25}$$

Where, B = number of strokes of pump applied in benign atmosphere; and S = number of strokes of pump applied in atmosphere under study.

7.2.3. Carbon monoxide

The main sources of carbon monoxide (CO) in air are gasoline engines and burning of coal. It is an important gaseous pollutant injurious to health. It combines about 300 times faster than does oxygen with haemoglobin in blood to form carboxyhaemoglobin which impairs normal oxygen transport by blood. Low level of CO poisoning may produce symptoms like reduction in reaction time, psychomotor impairment, headache, dizziness, and lassitude. At high level it may cause nausea, heart palpitations, difficulty in breathing, and even death.

Modern infrared gas analyser made for determination of specific gas is the most convenient and reliable device to measure CO concentration in air. However, a broad spectrum infrared analyser (spectrometer) may also be used for this purpose but then for different gases in mixture appropriate wavelength will have to be set.

The other technique of CO determination involves conversion of CO to methane in a reactor. The methane is then determined by gas chromatograph, in which it is mixed with an inert carrier gas and is slowly percolated through a column of silica gel or charcoal that selectively retards the passage of various gases in the mixture. From the bands on the column the content of gas in question is determine. The time for analysing each batch of air sample is about 20 minutes.

7.2.4. Nitrogen dioxide

The nitrogen dioxide (NO_2) is released in air from burning of fossil fuel in automobiles and power plants, and by the action of ultraviolet rays in the atmosphere it is reduced to nitrogen monoxide (NO) and atomic oxygen. Atomic oxygen reacts with oxygen to form ozone.

$$NO_2 \xrightarrow[\text{reduction}]{\text{Photochemical}} NO + O$$

$$O_2 + O \longrightarrow O_3$$

The atomic oxygen in the presence of sun light may also react with reactive hydrocarbons (e.g. ethane, methane, toluene etc., present in the atmosphere mainly as a result of burning of fossil fuel) to form reactive intermediates known as radicals. The radicals undergo a series of reactions to form still more radicals and as a result secondary pollutants such as formaldehyde and peroxyacetyl nitrate (PAN), are formed, collectively known as **photochemical smog**.

Oxides of nitrogen are pungent, causing irritation of eye and nose, and pulmonary ailments. The secondary pollutants, as mentioned above, are also harmful to both animals and plants.

The nitrogen monoxide and nitrogen dioxide can best be determined by using an analyser based on the principle of infrared lasers.

7.2.5. Sulphur dioxide

Sulphur dioxide (SO_2) is one of the common gaseous pollutants. It is released in air mainly from combustion of petroleum and coal. The pollutant is quite injurious to health as it causes irritation of eyes and respiratory epithelium, pharyngitis, cough, headache, and breathing troubles. In the atmophere SO_2 reacts with moisture to form sulphuric acid which may precipitate as **acid rain** — injurious to human health, plant life, and cultural assets.

I. Conductimetric method

In this method a known volume of air is bubbled through a dilute solution of sulphuric acid and hydrogen peroxide. Sulphur dioxide present in the air is oxidised to sulphuric acid, thereby increasing the electrical conductivity of the solution which can be measured and converted to the SO_2 content per unit volume of air.

II. Electrometric method

The air sample is bubbled through an electrolyte solution containing 0.1 M of potassium bromide in 2N sulphuric acid. The SO_2 contained in air causes a decrease in bromine concentration in solution which is indicated by electrode current and can be measured electrometrically and related to SO_2 content of air. This method is suitable for continuous monitoring of SO_2 in air.

III. West-Gaeke method (Pararosaniline method)

A known volume of air is bubbled through 0.1 N solution of sodium tertachloromerculate which absorbs SO_2 present in air and prevents its oxidation to SO_3. The solution is then reacted with formaldehyde and bleached pararosaniline resulting in the formation of a reddish-purple colour which can be measured photometrically and SO_2 is thus calculated. This method permits determination of SO_2 in air in a wide range of concentration (from 0.002 to 5 ppm).

IV. Flame emission method

This method allows measurement of total sulphur (as SO_2, SO_3, H_2S, and mercaptans) in air. The air sample is mixed with an excess of hydrogen and burned in a flame photometer. The emittance is measured at about 0.394 µ and total sulphur is calculated per unit volume of air.

7.2.6. Hydrocarbons

The chief sources of hydrocarbons (such as ethane, methane, toluene etc.) in air are burning of fossil fuel and emissions from chemical industry. These combine with other chemicals, like nitrogen, to form secondary pollutants and give rise to photochemical smog. Hydrocarbons in air are hazardous to animal as well as plant life.

The common method employed for hydrocarbon estimation is the flame ionisation method. The air sample contaminated with hydrocarbons is burned with excess of hydrogen in a flame. A pair of electrodes is maintained in the flame across which a potential difference of several hundred volts is developed. The resulting current in the electrode circuit is mesured which is proportional to the number of carbon atoms present. The value is converted into total hydrocarbons per unit volume of air. This method, however, underestimtes the oxygenated hydrocarbons of low molecular weight.

7.3. BIOLOGICAL PARAMETERS

Birds and insects may spend a part of their life in air but there is no organism which has an exclusive aerial mode of life. Some bacteria and pollens and spores of plants may occur as suspended in air.

7.3.1. Bacteria (Standard Plate Count or SPC)

Bacteria in the air are associated with dust, droplets, and nasal and salivary secretions. They are also carried away from soil with wind. Many of such bacretia may be pathogenic, so this parameter is of great concern to human health. The atmosphere of operation theatres, drug and food industry etc. should, therefore, be kept bacteria-free as far as possible.

Material

All material as required for making SPC in water (see 3.3.6-1).

Method

Take the nutrient agar medium (see 3.3.6-1) in sterilized Petri plates of 10 cm diameter. Expose the plates to the atmosphere under investigation for 1 minute and transfer the plates to incubator where these are incubated at 37 °C for 24 hours. On completion of the incubation period count the bacteria colonies. Because during exposure the bacteria carrying particles of only larger size settle on the plates this method gives only a bacteriological approximation for air in question.

For quantitative analysis pass a known volume of air in Petri plates containing nutrient agar medium and covered by an aluminium foil having a slit. After incubation count the colonies and express the result as number of colonies per unit volume of air.

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APPENDICES

Appendix I. Some useful equivalents.

Length: 1 **A°** = 10^{-1} **nm** = 10^{-4} **µm** = 10^{-7} **mm** = 10^{-8} **cm** = 10^{-10}**m** = 10^{-13} **km**

Volume: 1 **ml** = 1 **cm³** = 10^{-3} **l** = 10^{-3}**dm³** = 10^{-6} **m³**

Area: 1 **cm²** = 10^{-2} **dm²** = 10^{-4} **m²** = 10^{-6} **dam² (are)** = 10^{-8} **ha** = 10^{-10} **km²**

Mass: 1 **g** = 10^{3} **mg** = 10^{6} **µg**

Pressure: 1 **Pa** (Pascal) = 1 **N/m²** = 10 **dyn/cm²** = 1.01972 x 10^{-5}**kg/cm²** = 10^{-5} **bar**

Mass concentration: 1 **µg/ml** = 1 **mg/l** = 1 **mg/dm³** = 1 **g/m³** = 1 **ppm**

1 **µg/l** = 1 **µg/dm³** = 1 **mg/m³** = 1 **ppb**

Appendix II. Factors to convert meq/l to mg/l and vice versa.

Ions	meq/l = mg/l x	mg/l = meq/l x
Ca^{++}	0.04990	20.04
Mg^{++}	0.08224	12.16
Na^{+}	0.04350	22.99
K^{+}	0.02558	39.10
HCO_3^{-}	0.01639	61.02
$CO_3^{=}$	0.03333	30.01
$SO_4^{=}$	0.02082	48.03
Cl^{-}	0.02820	35.46

Appendix III. Some indicators and their pH ranges.

Indicator	pH range	Colour	
		Acid	Base
Methyl orange	2.8-4.0	Red	Orange
Bromophenol blue	3.0-4.6	Yellow	Blue
Bromocresol green	3.6-5.2	Orange	Blue-green
Methyl red	4.2-6.3	Red	Yellow
Bromothymol blue	6.0-7.6	Yellow	Blue
Phenol red	6.8-8.4	Yellow	Red
Thymol blue	8.0-9.6	Yellow	Blue
Phenolphthalein	8.3-10.0	Colourless	Red

Appendix IV. Mesh size (μ) of different types of bolting cloth.

No.	Silk gauze	Monodur perlon	Monofilament nylon
0	490	500	500
3	285	280	300
8	195	200	180
12	106	112	112
16	74	71	87
20	63	63	75
25	55	56	65
20GG	1000	1000	–
30GG	670	670	–
53GG	328	335	–

Appendix V. Dinking water standards as prescribed by World Health Organization, 1971

Characteristics	Highest desirable	Maximum permissible
PHYSICAL		
Turbidity (JTU)	5.00	25.00
Taste and odour	Nothing disagreeable	
Colour(Pt. scale)	5.00	50.00
CHEMICAL		
pH (units)	7.0-8.5	6.5-9.2
Total solids	500.00	1500.00
Total hardness (as $CaCO_3$)	100.00	500.00
Magnesium	30.00	150.00
Iorn (Fe)	0.10	1.00
Manganese	0.05	0.50
Copper	0.05	1.00
Chloride	200.00	600.00
Sulphates (as SO_4)	200.00	400.00
Phenolic substances	0.001	0.002
Fluoride*	1.00	1.50
Nitrate	45.00	45.00
Zinc	5.00	15.00
Mineral oil	0.01	0.30
Anionic detergents (as MBAS)	0.20	1.00
Arsenic	-	0.05
Hexavalent Chromium	-	0.01
Cyanide	-	0.05
Lead	-	0.10
Selenium	-	0.01
Cadmium	-	0.01
Mercury	-	0.001
PCB (µg/l)	-	0.20
Gross Alfa-activity (PCi/l)	-	3.00
Gross Beta-activity (PCi/l)	-	30.00

BACTERIOLOGICAL

A. WATER ENTERING DISTRIBUTION SYSTEM: If disinfected, coliform count in any sample of 100 ml should be zero.

B. WATER IN THE DISTRIBUTION SYSTEM: Ideally all samples taken fom the distribution system including consumer's premises should be free from coliform organisms. Since in practice it is not always possible, hence following standards:

i. throughout any year, 95% of the samples examined should not have any coliform organisms.

ii. **E. coli** count in 100 ml of any sample should be zero.

iii. Coliform organisms not more than 10 per 100 ml shall be present in any sample.

iv. Coliform oganisms should not be detectable in 100 ml of any two consecutive samples.

All the values are in mg/l, unless otherwise stated.

* The acceptable Fluoride concentration varies as a function of ambient temperature.

Appendix VI. Drinking water standard as prescribed by Ministry of Works and Housing, 1975

Characteristics	Highest	Maximum
PHYSICAL		
Turbidity (JTU)	2.50	10.00
Taste and odour	Nothing disagreeable	
Colour (Pt. scale)	5.00	25.00
CHEMICAL		
pH (units)	7.0-8.5	6.5-9.2
Total solids	500.00	1500.00
Total hardness (as $CaCO_3$)	200.00	600.00
Magnesium	30.00	150.00
Iron (Fe)	0.10	1.00
Manganese	0.05	0.50
Copper	0.05	1.50
Chloride	200.00	1000.00
Sulphate (as SO_4)	200.00	400.00
Phenolic substances	0.001	0.002
Fluoride*	1.00	1.50
Nitrate	45.00	45.00
Zinc	5.00	15.00
Mineral oil	0.01	0.30
Anionic detergents (as MBAS)	0.20	1.00
Arsenic	0.05	0.05
Hexavalent Chromium	0.05	0.05
Cyanide	0.05	0.05
Lead	0.10	0.10
Selenium	0.01	0.01
Cadmium	0.01	0.01
Mercury	0.001	0.001
PCB (µg/l)	0.20	0.20
Gross Alfa-activity (PCi/l)	3.00	3.00
Gross Beta-activity (PCi/l)	30.00	30.00

BACTERIOLOGICAL

Coliform count in any sample of 100 ml should be zero.

Water in the distribution system shall satisfy all the three criteria indicated below:

i. **E. coli** count in 100 ml of any sample should be zero.

ii. Coliform organisms not more than 10 per 100 ml shall be present in any sample.

iii. Coliform organisms should not be detectable in 100 ml of any two consecutive samples or more than 50% of the samples collected for the year.

All the values are in mg/l, unless otherwise stated.

*The acceptable Fluoride concentration varies as a function of ambient temperature.

Appendix VII. Standards for disposal of waste water as prescribed by Indian Standards Institute (ISI)

Characteristics	Discharge of sewage into inland surface water	Industrial waste water in		For inland surface water when used as raw water for public water supplies and bathing ghats
		Inland surface water	Public sewers	
1	2	3	4	5
BOD_5 (20°C)	20.0	30.0	500.0	3.0
COD	-	250.0	-	-
pH (units)	-	5.5-9.0	5.5-9.0	6.0-9.0
TSS	30	100	600	-
Temperature °C	-	40	45	-
Oil and Grease	-	10	100	-
Phenolic comp.	-	1.0	5.0	0.005
Cyanides (as CN)	-	0.2	2.0	0.01
Sulphide (as S)	-	2.0	-	-
Fluorides (as F)	-	2.0	-	-
Insecticides	-	0.0	-	0.0
Arsenic (as As)	-	0.2	-	0.2
Cadmium (as Cd)	-	2.0	-	-
Chromium (as Cr hexavalent)	-	0.1	2.0	0.05 (total Cr)

Appendix VII. continued

1	2	3	4	5
Copper	-	3.0	3.0	-
Lead	-	0.1	1.0	0.1
Mercury	-	0.01	-	-
Nickel	-	3.0	2.0	-
Selenium	-	0.05	-	0.05
Zinc	-	5.0	15.0	-
Chlorides (as Cl)	-	-	600	600
Sulphates	-	-	-	1000
% Sodium	-	-	60	-
α-emitters µc/ml	-	10^{-7}	-	10^{-9}
β-emitters µc/ml	-	10^{-6}	-	10^{-8}
MPN of coliform/ 100 ml (monthly average)	-	-	-	should not exceed 5000

All values in mg/l, unless otherwise stated.

Appendix VIII. Minimum National Acceptable Standards (MINAS) for the effluents of various industries. Values are upper limits of concentration of relevant parameters in mg/l, except pH or otherwise stated. [**Industries:** 1. Man-made Fibre - a. Synthetic Fibre, b. Semi-synthetic Fibre; 2. Cotton Textile (Composite processing); 3. Composite Woollen Mill; 4. Nitrogenous (straight) Fertilizer; 5. Phosphatic (straight) Fertilizer; 6. Complex Fertilizer (with/without straight nitrogenous & phosphatic fertilizer); 7. Sugar; 8. Fermentation Industry - a. Maltry, b. Brevery, c. Distillery; 9. Caustic Soda (Mercury cell)].

Industry		pH	Suspended solids	BOD_5	Zn	Oil/ grease	Bioassay test	TDS	%Na	Cr VI
1.	a	5.5-9.0	100	30	–	–	–	–	–	–
	b	5.5-9.0	100	30	1	–	–	–	–	–
2.		5.5-9.0	100	150	–	10	90%*	2100	60	–
3.		5.5-9.0	100	100	–	10	90%*	–	–	–
4.		6.5-8.0	100	–	–	10	–	–	–	0.1
5.		7.0-9.0	100	–	–	10	–	–	–	0.1
6.		6.5-8.0	100	–	–	10	–	–	–	0.1
7.		6.5-8.0	100	100	–	–	–	–	–	–
8.	a	–	100	30	–	–	–	–	–	–
	b	–	100	30	–	–	–	–	–	–
	c	–	–	30	–	–	–	–	–	–
9.		5.5-9.0	100	–	–	–	–	–	–	–

*survival after 96 h.

Appendix VIII. continued

Industry	Cr total	S	Phenolic compounds	NH_3-N	TKN	Free NH_3	NO_3-N	CN	As	F	Hg
1. a	–	–	–	–	–	–	–	–	–	–	–
b	–	–	–	–	–	–	–	–	–	–	–
2.	–	–	–	–	–	–	–	–	–	–	–
3.	2.0	2.0	1.0	–	–	–	–	–	–	–	–
4.	2.0	–	–	50	100	4.0	10	0.2	0.2	–	–
5.	2.0	–	–	–	–	–	–	–	–	10	–
6.	2.0	–	–	50	100	4.0	10	0.2	0.2	10	–
7.	–	–	–	–	–	–	–	–	–	–	–
8. a	–	–	–	–	–	–	–	–	–	–	–
b	–	–	–	–	–	–	–	–	–	–	–
c	–	–	–	–	–	–	–	–	–	–	–
9.	–	–	–	–	–	–	–	–	–	–	0.01

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